A CLASS-BOOK OF ORGANIC CHEMISTRY

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A CLASS-BOOK OF ORGANIC CHEMISTRY

BY

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FOR SECOND YEAR MEDICAL STUDENTS AND OTHERS

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PREFACE

THE object of this little volume is to furnish the ordinary medical student (who can only devote a very limited time in his second year to organic chemistry) with a short course on that portion of the subject which may be of use to him in his subsequent studies.

The plan which has been followed is the same as that described in the first volume of this class-book, namely, to combine the theoretical account with practical illustrations.

The latter have been selected and for the most part rehearsed with great care, so that in following the detailed directions the student should obtain the desired result without loss of time.

I am indebted to my friend Mr. P. K. Dutt for help in working out some of the experimental details.

J. B. COHEN.

THE UNIVERSITY,
LEEDS.

September, 1919.

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CHAPTER I

SYNTHESIS

THE term *synthesis* in organic chemistry is usually applied to the production by laboratory methods of naturally occurring products of animal and plant life. It is, however, often used in a wider sense to denote preparations which, like synthetic drugs (p. 128), possess properties similar to those of natural substances.

A great variety of reactions are involved in the process of synthesis; but it is doubtful if any one of them is actually utilised by the living organism. For laboratory methods are, as a rule, accompanied by a considerable absorption or evolution of heat, denoting violent chemical changes, whereas the living organism effects its results at the ordinary temperature, that is, with small energy changes and often with great rapidity. There is, for example, no laboratory method to compare with the rapid formation at the ordinary temperature of so complex a substance as starch from carbon dioxide. Although most of the simpler vital products have now been synthesised, those of great complexity (which are at the same time of commoner occurrence), such as starch, cellulose, and the proteins, still defy the skill of the chemist. Moreover, the vital processes whereby these substances are elaborated in the organism remain very obscure. They appear in general to be determined by the

intervention of enzymes or vital catalysts, to which reference will be made in a later chapter. Synthetic methods consist in linking together groups of atoms in which, as a rule, one atom in one molecule is linked to one in another molecule. Such a linking may be effected between carbon and carbon, carbon and nitrogen, or carbon and oxygen, either in the form of an open or a closed chain or ring. Whilst this process of linking is a fundamental part of the synthetic method, many of the more familiar chemical reactions such as oxidation, reduction, halogenation, nitration, sulphonation, etc., may be indirectly involved.

Carbon–Carbon chain formation (Condensation).—Condensation includes a variety of reactions in which carbon is made to combine with carbon, and is usually defined as the union of two or more organic molecules or parts of the same molecule (with or without the elimination of component elements), in which the new combination is effected between carbon atoms. For example, the combination of two molecules of acetaldehyde to form aldol or crotonaldehyde is a typical case of condensation. In the first reaction no elimination of elements occurs; in the second, in which a dehydrating agent is present, water is removed.

$$\underset{\text{Acetaldehyde.}}{CH_3.CHO} + \underset{\text{Acetaldehyde.}}{CH_3.CHO} = \underset{\text{Aldol.}}{CH_3.CH(OH).CH_2.CHO}$$

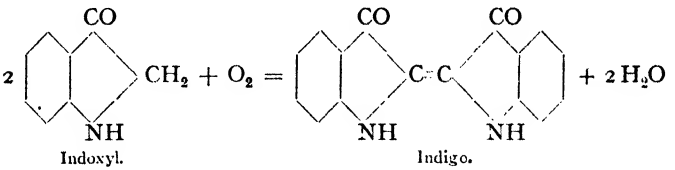
$$\underset{\text{Acetaldehyde.}}{CH_3.CHO} + \underset{\text{Acetaldehyde.}}{CH_3.CHO} = \underset{\text{Crotonaldehyde.}}{CH_3.CH{:}CH.CHO} + H_2O$$

Where two or more molecules are involved, as in the present instance, the process is called *external* condensation to distinguish it from the linking together of parts of the same molecule or *internal* condensation, as in the formation of cyclopropane, which is obtained by removing bromine by means of sodium or zinc from trimethylene bromide.

$$\underset{\text{Trimethylene bromide.}}{BrCH_2.CH_2.CH_2Br} + Zn = \underset{\text{Cyclopropane.}}{\begin{matrix} CH_2 \\ \diagup\diagdown \\ CH_2 \end{matrix}\!\!-\!\!\begin{matrix} \\ \\ CH_2 \end{matrix}} + ZnBr_2$$

Condensation may be effected by a variety of reactions, which we will now proceed to consider. They may be divided into methods of substitution and of addition.

Substitution methods. — 1. Condensation may occur through the removal of hydrogen by oxidation as in the formation of indigo from indoxyl.



2. By the removal of halogens by the action of metals, as in the synthesis of paraffins (Wurtz) and aromatic hydrocarbons (Fittig) (Vol. I, p. 76).

$$\underset{\text{Ethyl iodide.}}{2C_2H_5I} + 2Na = \underset{\text{Butane.}}{C_4H_{10}} + 2NaI$$

$$\underset{\text{Bromobenzene.}}{C_6H_5Br} + \underset{\text{Ethyl bromide.}}{C_2H_5Br} + 2Na = \underset{\text{Ethyl benzene.}}{C_6H_5{\cdot}C_2H_5} + 2NaBr.$$

EXPT. 1.—**Preparation of Ethyl benzene.**—Pour into a round flask (1 litre) 150 c.c. of ether which has been carefully purified and dehydrated over sodium (Vol. I, p. 39), and add 26·5 grams of metallic sodium in thin slices or fine wire. Attach the flask to a reflux condenser and immerse it in a vessel of ice-water. When all evolution of hydrogen has ceased introduce 60 grams of bromobenzene (Vol. I, p. 250) and 52 grams of ethyl bromide (Vol. I, p. 30), both carefully dehydrated. The reaction is allowed to commence spontaneously, the fact being indicated by the appearance of the sodium, which becomes darker in colour and sinks to the bottom of the vessel. The flask must not be removed from the water until the reaction is complete, and it is convenient to leave it overnight. The liquid is then decanted into a distilling flask from the sodium bromide (which has a blue colour) and is rinsed out once or twice with ether. The ether is removed by distillation from the water-bath, a bit of porous pot being added. The residue is then fractionated with a fractionating column and the portion boiling at 132–135° separately collected. The yield is 20–25 grams. Ethyl benzene is a colourless liquid : b.p. 134° and sp. gr. 0·8664 at 22·5°.

3. By the replacement of a halogen by the cyanogen group using potassium or sodium cyanide, and thereby introducing a new carbon atom which can be hydrolysed to the carboxyl group or reduced to a primary amine. Benzyl chloride and

potassium cyanide yield benzyl cyanide, which, on hydrolysis, is converted into phenyl acetic acid.

$$C_6H_5.CH_2Cl + KCN = \underset{\text{Benzyl cyanide.}}{C_6H_5.CH_2.CN} + KCl$$

$$C_6H_5.CH_2.CN + 2H_2O = \underset{\text{Phenyl acetic acid.}}{C_6H_5.CH_2.COOH} + NH_3$$

When reduced, benzyl cyanide gives phenyl ethylamine

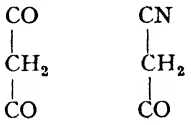
$$C_6H_5.CH_2.CN + 2H_2 = \underset{\text{Phenyl ethylamine}}{C_6H_5.CH_2.CH_2.NH_2}$$

EXPT. 2.—**Preparation of Phenyl acetic acid.**—Dissolve 30 grams of *pure* potassium cyanide in 25 c.c. of water in a round flask (500 c.c.) attached to a reflux condenser. Heat on the water-bath, and to the hot solution pour in slowly through the top of the condenser 50 grams of benzyl chloride dissolved in an equal weight of alcohol. Boil gently for three to four hours in the fume cupboard. The liquid separates into two layers; the upper brown layer consisting of an alcoholic solution of benzyl cyanide and the lower of an aqueous solution of potassium chloride. The upper layer is separated and fractionally distilled. Alcohol first distils, a little water follows and, when the temperature reaches 210°, the distillate consists of benzyl cyanide. It is collected between 210° and 235°. The yield is about 35 grams. It is hydrolysed with three times its weight of a mixture of 3 vols. of conc. sulphuric acid and 2 vols. of water. The mixture of cyanide and acid are introduced into a round flask (500 c.c.) which is connected, by a glass tube bent twice at right angles, with a Woulff bottle. The end of this tube passes just beneath the cork in the one tubulus of the bottle which contains water. Through the second tubulus a 100 c.c. pipette is fitted vertically and dips just below the surface of the water. The flask containing the mixture (which divides into two layers) is heated over the naked flame, gently at first; the temperature is then raised until small bubbles are observed to rise from the surface of the lower layer of acid. The flame is now removed and in a few minutes a vigorous reaction sets in; the liquid boils and a small quantity of benzyl cyanide distils into the Woulff bottle. At the same time the water is forced up the pipette, which serves as a valve. The reaction is complete in a minute; the flask is heated again for two to three minutes and then allowed to cool. On standing the liquid solidifies to a laminated, crystalline mass. It is purified by washing with cold water. The crystals are dissolved in water, neutralised with sodium

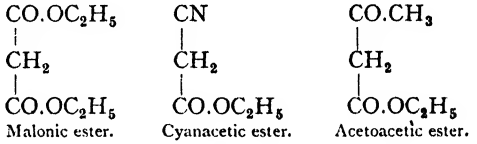
carbonate solution, the hot solution filtered and acidified dilute sulphuric acid. On standing, colourless plates of the acid separate and are filtered and dried; m.p. 76–77°.

$$C_6H_5.CH_2.CN + 4H_2O + H_2SO_4 = 2C_6H_5.CH_2.COOH + (NH_4)_2SO_4$$

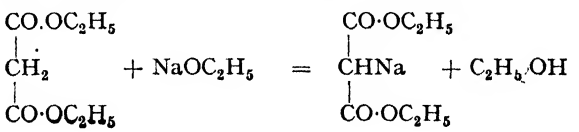
4. The reverse process of that described under method 2 can employed, in which the metal derivative of an organic compoun is removed by the action of a halogen or halogen derivative. There are a number of organic compounds containing the groups:



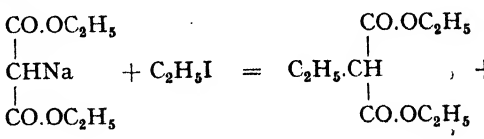
in which the central hydrogen atoms are replaceable by sodium. Such substances as malonic ester, cyanacetic ester and acetoacetic ester belong to this category.



Thus, by adding a solution of sodium ethoxide to malonic ester a mono- or di-sodium derivative is formed which reacts with alkyl iodides to form mono- and di-alkyl derivatives:



By the action of an alkyl halide, the sodium is replaced by the alkyl group:



EXPT. 3.—**Preparation of Ethyl malonic acid.**—Dissolve 2·3 grams of sodium in 75 grams of absolute alcohol in a round flask

c.c.) fitted to a reflux condenser. Whilst the solution is warm add 16 grams of malonic ester[1] from a tap-funnel inserted through the top of the condenser. The liquid remains clear at first, but before the ester has all been added sodium ethyl malonate begins to crystallise and forms a semi-solid mass. To this 20 grams of ethyl iodide (Vol. I, p. 30) are slowly added. The mass softens and, after continued shaking, completely liquefies with evolution of heat. The product is now heated on the water-bath, when it becomes turbid from the separation of sodium iodide. After one to two hours the liquid ceases to be alkaline to litmus and the reaction is complete. The alcohol is distilled from a brine-bath (water saturated with common salt). On the addition of water to the residue an almost colourless oil separates. The oil is removed by extraction with ether, dehydrated over calcium chloride, decanted and distilled. When the ether has been removed almost the whole of the ethyl malonic ester passes over at 206–208°. The yield should be about 15 grams. It is a colourless liquid with a fruity smell. To obtain the free acid, the ester is hydrolysed with potassium hydroxide. Make a strong solution of the alkali by dissolving 15 grams of potassium hydroxide in 50 c.c. of water. To the solution add slowly 10 grams of the ester. An emulsion forms which soon solidifies to a colourless mass. Heat the mixture on the water-bath with frequent shaking for about three-quarters of an hour, until it becomes completely liquid, when the hydrolysis is complete. The product is acidified with conc. hydrochloric acid, and the free ethyl malonic acid is extracted by shaking with ether. The ethereal solution is dehydrated over anhydrous sodium sulphate and decanted into a distilling flask. After distilling off the ether, the acid remains as a syrup, which solidifies on standing. It is redissolved in water, boiled with a little animal charcoal to free it from adhering colouring matter, filtered and evaporated to a syrupy consistency on the water-bath. The yield of acid is about 5 grams which melt at 112°. Heat about a gram of the acid in a test-tube over a small flame and have at hand a second test-tube one-third full of lime-water. The acid decomposes with evolution of carbon dioxide into butyric acid which is perceived by its rancid smell, whilst the presence of carbon dioxide is detected by decanting the gas into lime water:

$$\underset{\text{Ethyl malonic acid.}}{C_2H_5.CH(CO_2H)_2} = \underset{\text{Butyric acid.}}{C_3H_7.COOH} + CO_2$$

[1] The preparation of malonic ester is described in Cohen's *Practical Organic Chemistry*. Macmillan & Co.

A second sodium atom and a second alkyl group may be introduced by a repetition of the above process, or, directly, by using two molecules of sodium alcoholate.

$$\begin{array}{l} CO.OC_2H_5 \\ | \\ CH_2 \\ | \\ CO.OC_2H_5 \end{array} + 2NaOC_2H_5 \qquad \begin{array}{l} CO.OC_2H_5 \\ | \\ CNa_2 \\ | \\ CO.OC_2H_5 \end{array} + 2C_2H_5.OH$$

$$\begin{array}{l} CO.OC_2H_5 \\ | \\ CNa_2 \\ | \\ CO.OC_2H_5 \end{array} + 2C_2H_5I \qquad \begin{array}{l} CO.OC_2H_5 \\ | \\ C(C_2H_5)_2 \\ | \\ CO.OC_2H_5 \end{array} + 2NaI$$

Diethylmalonic ester.

Diethyl malonic ester is used in the synthesis of **veronal** (p. 18). On hydrolysis and by heating the free acid, diethylacetic acid is obtained.

$$(C_2H_5)_2C(COOH)_2 = (C_2H_5)_2CH.COOH + CO_2.$$

In this way a variety of dibasic and monobasic aliphatic acids can be prepared (see p. 81).

5. By the removal of halogen acid in which hydrogen is eliminated from one molecule, which must be an aromatic hydrocarbon, and halogen from the other (Friedel-Crafts' reaction). The agent here is anhydrous aluminium chloride, which acts as a catalyst. Toluene can be prepared from benzene and methyl chloride; acetophenone (phenyl methyl ketone) from benzene and acetyl chloride:

$$C_6H_6 + CH_3Cl + [AlCl_3] = \underset{\text{Toluene.}}{C_6H_5.CH_3} + HCl$$

$$C_6H_6 + CH_3.COCl + [AlCl_3] = \underset{\text{Acetophenone.}}{C_6H_5.CO.CH_3} + HCl$$

EXPT. 4.—**Preparation of Acetophenone.**—Attach a round flask (500 c.c.) to a reflux condenser and bring into it 50 grams of anhydrous aluminium chloride which should be freshly prepared or recently sublimed in a current of HCl gas. Cover it immediately with 35 c.c. of benzene. Place the flask in ice-water and add 35 grams of acetyl chloride (Vol. I, p. 62) drop by drop from a tap-funnel inserted through the top of the condenser. A vigorous effervescence occurs, and dense fumes of hydrogen chloride are evolved. The contents of the flask are converted into a brown, viscid mass, which, after standing an hour, is stirred

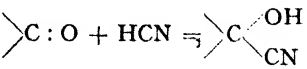
and shaken into a beaker containing ice and water (250 c.c.) and the flask rinsed with cold water. The mass decomposes with evolution of heat and a dark oil separates on the surface. The liquid is poured into a separating-funnel and a little benzene added. The aqueous portion is drawn off and the benzene layer shaken up with dilute caustic soda and then with water. The benzene solution is finally separated, dehydrated over calcium chloride, filtered, and distilled. The benzene first passes over; the thermometer then rises quickly to 195°. The receiver is now changed, the water run out of the condenser and the distillate, which boils at 195–200°, collected separately. It forms a pale yellow oil with a characteristic sweetish smell and solidifies completely on standing. The yield is 20–25 grams: m.p. 20°; b.p. 202°.

6. By the removal of carbon dioxide as calcium carbonate in the formation of ketones (Vol. I, p. 66) by heating the calcium salts of organic acids. Calcium benzoate and calcium acetate give acetophenone.

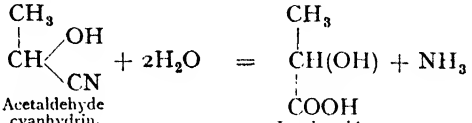
$$\begin{matrix} C_6H_5.COOca' \\ + \\ CH_3.COOca' \end{matrix} = \begin{matrix} C_6H_5 \\ \\ CH_3 \end{matrix}\!\!>\!CO + CaCO_3. \quad ca' = \frac{Ca}{2}$$

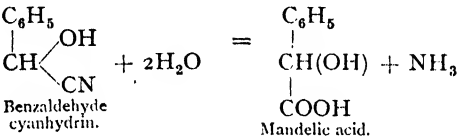
Expt. 5.—**Preparation of Acetophenone.**—Grind together in a mortar 100 grams of anhydrous calcium benzoate and 50 grams of dry calcium acetate. (The calcium salts are prepared by dissolving the acids in boiling water and adding milk of lime till alkaline. The solutions are filtered from any excess of lime and evaporated to crystallisation. The salts are dried and powdered and heated in a hot air oven at 150° until moisture ceases to condense on the cold surface of a watch glass held close above the substance). Introduce the mixture into an iron tube closed at one end and attached by a long air condenser to a receiver and heat the tube strongly in a combustion furnace, beginning at the open end. A brown oil distils. The distillate is dehydrated over calcium chloride, decanted, and fractionated. A small quantity of a colourless liquid first distils, when the temperature rises quickly to 195°, and the product, boiling at 195–205°, is separately collected. This is redistilled and collected at 198–202°. It crystallises on cooling.

Additive reactions.—7. One of the simplest additive reactions is the formation of cyanhydrins by the union of hydrogen cyanide with an aldehyde or ketone (Vol. I, p. 52).



These compounds yield, on hydrolysis, the corresponding hydroxy-acid. Thus, lactic acid was synthesised from acetaldehyde-cyanhydrin and mandelic acid from the corresponding benzaldehyde compound.

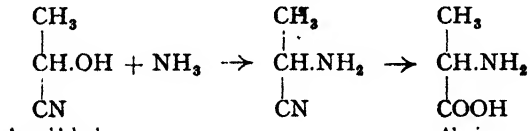




EXPT. 6.—**Preparation of Mandelic acid.**—Fifteen grams of freshly distilled benzaldehyde are mixed with 50 c.c. of a saturated solution of sodium bisulphite. (The latter is prepared by covering 25–30 grams of powdered, crystallised sodium carbonate with a thin layer of water, and passing in sulphur dioxide until the crystals are dissolved and an apple-green solution is formed.) The mixture forms a semi-solid mass of the bisulphite compound which after standing for half an hour is filtered and pressed at the pump and washed with a little water and spirit. The mass is then ground into a thick paste with water, and a solution of 12 grams of pure potassium cyanide or 8 grams of sodium cyanide added. After a short time the cyanhydrin separates as a reddish oil, to which a little ether is added and then removed by means of a tap-funnel. The ether is allowed to evaporate on the water-bath and the product hydrolysed by continuing to heat on the water-bath with the addition of four to five times its volume of conc. hydrochloric acid until crystals appear on the surface. Water is added and the hot liquid decanted and filtered from any oil. On cooling, the crystals are filtered, washed with a little cold water, and dried. A further quantity can be extracted from the filtrate with ether. Yield 10–15 grams. Colourless needles: m.p. 118–119°.

By conducting this reaction in presence of ammonia the corresponding amino-acid can be prepared. Alanine (amino-

propionic acid was first synthesised from acetaldehyde in this way:



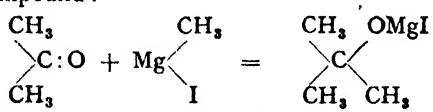
and leucine from isovaleric aldehyde (p. 85).

8. There are many organo-metallic compounds which combine directly with the unsaturated part of a second organic molecule. Such reactions are those of Grignard and Reformatsky. The **Grignard reagent**, as it is called, is prepared by dissolving magnesium in an ethereal solution of an alkyl or aryl[1] bromide or iodide which form substances of the general formula:

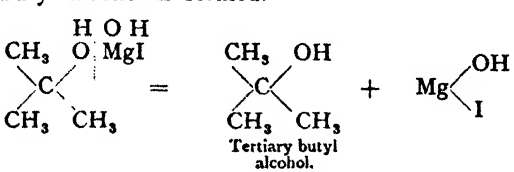
R′ = alkyl or aryl radical

X = Br, I.

This reagent forms additive compounds with aldehydes, ketones, carbon dioxide and cyanides, and further by substitution as well as addition, with esters. The following examples will serve to illustrate the process. In each case the same positive metallic atom (Mg) attaches itself to the more negative element (O or N). Acetone and magnesium methyl iodide give the following compound:



On decomposing the product with water or dilute sulphuric or hydrochloric acid to dissolve the magnesium oxyiodide, tertiary butyl alcohol is formed.



[1] The term *aryl* is given to the aromatic univalent radical such as C_6H_5', $(CH_3)C_6H_4'$, etc.

With benzaldehyde and magnesium methyl iodide, phenyl methyl carbinol is produced:

$$C_6H_5.CHO + Mg\begin{matrix}CH_3\\I\end{matrix} \rightarrow C_6H_5.CH.\begin{matrix}OMgI\\CH_3\end{matrix} \rightarrow$$

$$C_6H_5.CH(OH).CH_3 + Mg(OH)I$$

Phenyl methyl carbinol.

EXPT. 7.—(*a*) **Preparation of Tertiary Butyl alcohol** (Grignard).—The magnesium methyl iodide is first prepared as follows: Place 6 grams of clean magnesium ribbon or filings in a dry, round flask (1 litre) connected with a long condenser and dropping funnel as shown in Fig. 1. Thirty-six grams of methyl iodide and 50 c.c. of ether, previously well dehydrated over metallic sodium, are mixed in a separate vessel, and 20 c.c. of this mixture poured on to the magnesium. In a few seconds a vigorous reaction usually sets in or, if it is delayed, may be started by adding a crystal of iodine. When the first reaction has subsided, add 70 c.c. of dry ether and run in the remainder of the alkyl iodide and ether mixture drop by drop from a tap-funnel. The contents of the flask are then boiled on the water-bath for a short time until the magnesium is dissolved. The flask is now surrounded by a freezing mixture of powdered ice and salt and 12 grams of acetone are added drop by drop. An energetic action takes place; each drop of acetone produces a hissing sound and forms a white precipitate. After the acetone has been added, the mixture is left for a few hours, and carefully decomposed in the cold by the gradual addition of dilute sulphuric acid. The ethereal solution is removed and the aqueous portion distilled in steam to remove dissolved alcohol until about 100 c.c. have distilled. The distillate is saturated with potassium carbonate, extracted with ether and the ether solution added to the previous portion. The whole ether solution is dehydrated over solid potassium carbonate and fractionated slowly on the water-bath until the thermometer reaches 40°. The residue, which is a hydrate of the alcohol, is heated with 4 grams of

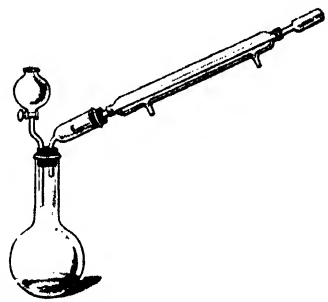


FIG. 1.

barium oxide with reflux for an hour and then distilled to 100° from an oil-bath. The distillate is refractionated and collected at 81–83°. Yield 8–10 grams.

(*b*) **Preparation of Phenyl methyl carbinol** (Grignard).—The first half of the process for the preparation of the magnesium methyl iodide (*Grignard reagent*) is described above. When the magnesium is dissolved immerse the flask in ice-water, and add 26 grams of benzaldehyde mixed with an equal volume of dry ether from the tap-funnel, with constant shaking. The solid magnesium compound separates and is left over-night. The flask is then cooled in ice-water and sufficient dilute sulphuric or hydrochloric acid added drop by drop to dissolve the magnesium compound. Remove the aqueous layer in a separating-funnel and wash the ether, first with sodium bicarbonate solution, then with sodium bisulphite (to remove free iodine) and again with sodium bicarbonate. Dry the ether extract over potassium carbonate and remove the ether by distillation on the water-bath. The phenyl methyl carbinol which remains is distilled under reduced pressure (Vol. I, p. 277): b.p. 100° at 15 mm.; 110–111° at 28 mm.; 118° at 40 mm. Yield is 20 grams.

In the case of a cyanide, the following type of reaction occurs, whereby methyl cyanide is converted into acetone.

$$CH_3.C{:}N + Mg\begin{matrix}CH_3\\ I\end{matrix} = CH_3.C\begin{matrix}NMgI\\ CH_3\end{matrix}$$

$$CH_3.C\begin{matrix}NMgI\\ CH_3\end{matrix} + 2H_2O = CH_3.CO.CH_3 + Mg(OH)I + NH_3$$

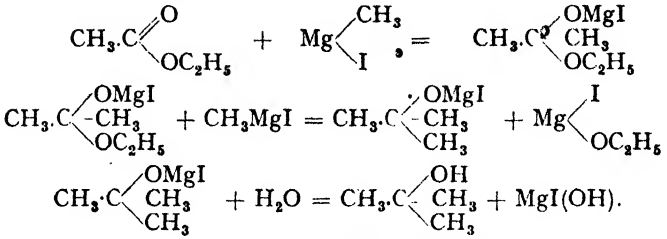
The product in this case is a ketone.

By passing carbon dioxide into an ethereal solution of the reagent and decomposing the product with water an acid is obtained:

$$CH_3MgI + CO_2 \rightarrow CH_3.C\begin{matrix}OMgI\\ O\end{matrix} \rightarrow CH_3.C\begin{matrix}OH\\ O\end{matrix} + MgI(OH).$$

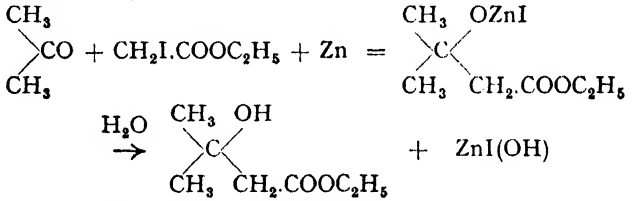
Acetic acid.

Esters react as follows, giving a tertiary alcohol. Ethyl acetate yields tertiary butyl alcohol.

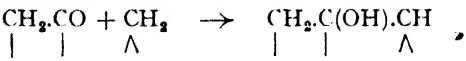


Tertiary butyl alcohol.

Reformatsky's reaction is merely a variation of the above, in which the metal (Zn or Mg) acts upon a mixture of ketone and halogen compound thus:

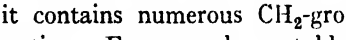


Aldol condensations.—9. The following group of reactions, termed for brevity **aldol condensations**, are among the most important of the condensation methods. They consist in the union of two organic compounds, one of which contains the group CH_2, and the second the group $CH_2.CO$, the combination being brought about, as in the formation of aldol, by the shifting of a hydrogen atom from the CH_2 group of the first compound to the oxygen of the second.



The process is, however, subject to certain restrictions. A paraffin, although it contains numerous CH_2-groups, does not undergo this condensation. For example, acetaldehyde does not unite with methane unless a negative group, CO, CN, NO_2, replaces at least one hydrogen atom in the paraffin.

Aldol condensation.—This consists in the union of two aldehyde molecules in presence of an alkali as already explained (p. 2).



By the elimination of water, crotonaldehyde is formed. This occurs if zinc chloride is allowed to react with acetaldehyde or aldol.

$$\underset{\text{Aldol.}}{CH_3.CH(OH).CH_2.CHO} = \underset{\text{Crotonaldehyde.}}{CH_3.CH{:}CH.CHO} + H_2O.$$

Two ketones react in a similar fashion. Acetone gives mesityl oxide and phorone.

$$\begin{matrix} CH_3 \\ \diagdown \\ \quad CO \\ \diagup \\ CH_3 \end{matrix} + CH_3.CO.CH_3 = \begin{matrix} CH_3 \\ \diagdown \\ \quad C{:}CH.CO.CH_3 \\ \diagup \\ CH_3 \end{matrix} + H_2O$$

Mesityl oxide.

$$\begin{matrix} CH_3 \\ \diagdown \\ \quad CO \\ \diagup \\ CH_3 \end{matrix} + CH_3.CO.CH_3 + OC\begin{matrix} CH_3 \\ \diagup \\ \diagdown \\ CH_3 \end{matrix} =$$

$$\begin{matrix} CH_3 \\ \diagdown \\ \quad C{:}CH.CO.CH{:}C \\ \diagup \\ CH_3 \end{matrix}\begin{matrix} CH_3 \\ \diagup \\ \diagdown \\ CH_3 \end{matrix} + 2H_2O$$

Phorone.

EXPT. 8.—**Preparation of Mesityl oxide and Phorone.**—Two hundred and fifty c.c. of acetone are dehydrated by standing overnight with calcium chloride and then distilled. The acetone is poured into a bottle (1 litre) provided with a double bored cork. Through one hole a delivery tube passes to the bottom of the vessel and through the other a calcium chloride tube is inserted. The acetone, cooled in a freezing mixture, is saturated with hydrogen chloride, which is evolved by dropping conc. sulphuric acid from a tap-funnel on to conc. hydrochloric acid. With a rapid stream the process may take two to three hours, and the increase in weight is about 60 per cent. After saturation, the bottle is left in ice or ice-water for twenty-four hours, and then at the ordinary temperature for two days. The dark coloured liquid is now poured on to about 300 grams of crushed ice and well stirred. The upper layer, which contains the mesityl oxide, is separated and shaken with a strong solution of caustic soda until faintly yellow. Thus purified it is distilled in steam with the addition of a little strong caustic soda solution to decompose any hydrochloride of mesityl oxide which may remain. The distillate is separated, dehydrated over calcium chloride, and finally fractionated with a column, first on the water-bath and then over the flame. A small quantity distils below 65°,

the temperature then rises and the portions boiling at 129–107° and 180–200° are collected separately. The lower boiling fraction is re-fractionated between 129° and 131° and consists of mesityl oxide. Phorone, which constitutes the higher boiling portion, boils at 190–191°. The yield is about 100 grams of mesityl oxide; b.p. 130°; sp. gr. 0·848 at 23°. Phorone crystallises in yellowish-green crystals, m.p. 28°; b.p. 190–191°.

Acetoacetic ester condensation.—Here the two aldehyde or ketone groups are replaced by two ester groups. The condensing agent in this case is metallic sodium or sodium ethoxide. Ethyl acetate in presence of metallic sodium yields acetoacetic ester.

$$CH_3.COOC_2H_5 + CH_3.COOC_2H_5 + Na \longrightarrow$$

$$CH_3.C(ONa)(OC_2H_5)CH_2.COOC_2H_5 + H$$

Intermediate compound.[1]

$$\longrightarrow CH_3.C(ONa){:}CH.COOC_2H_5 + C_2H_5.OH.$$

Sodium acetoacetic ester.

$$CH_3.C(OH){:}CH.COOC_2H_5 \rightleftharpoons CH_3.CO.CH_2.COOC_2H_5.$$

Tautomeric forms of acetoacetic ester (see below).

EXPT. 9.—**Preparation of Acetoacetic ester.**—Pour 200 grams of carefully dehydrated ethyl acetate into a round flask (500 c.c.) connected with a reflux condenser. Add 20 grams of sodium in thin slices or wire and cool the flask in water. After a short time a brisk reaction sets in and ultimately the liquid boils. When the action slackens the flask is heated on the water-bath until the sodium is completely dissolved. A 50 per cent: acetic acid solution is at once added until the liquid is acid (about 100 c.c.) (well shaken) and then an equal volume of concentrated brine. The liquid divides into two layers; the upper one, consisting of acetoacetic ester and unchanged ethyl acetate, is carefully separated and distilled over wire-gauze until the thermometer marks 100°. The distillate is now collected in fractions, that distilling at 175–185° is nearly pure acetoacetic ester. Yield 30–40 grams. A better yield is obtained if the fractionation is conducted under reduced pressure (*see* Vol. I, p. 277).

Tautomerism.—Such substances as that obtained in the above reaction and which contain the groups

$$-CO.CH_2.CO- \text{ and } -CO.CH_2.CN$$

[1] The intermediate compound has not been isolated.

form, as already stated, sodium compounds. The structure of these compounds is, however, different from that of the original substance in that the metallic atom is attached to oxygen and not to carbon, thus :

$$C(ONa):CH.CO\ ;\ C(ONa):CH.CN$$

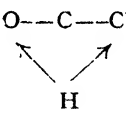
From the sodium derivative, acids in some cases release the corresponding compound,

$$C(OH):CH.CO\ ;\ C(OH):CH.CN$$

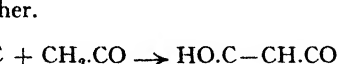
which possesses the properties of an unsaturated acid, and is termed the **enolic** form. It passes more or less readily into the **ketonic** form.

$$\underset{\text{Enolic.}}{C(OH):CH.CO} \rightleftharpoons \underset{\text{Ketonic.}}{CO.CH_2.CO}$$

These changes are therefore reversible, the phenomenon being termed **dynamic isomerism** or **tautomerism.** It may be explained by the wandering of a hydrogen atom from one polyvalent atom (O) to another (C) accompanied by a change of linkage.



Such isomeric changes are not uncommon and may serve to account for the aldol condensation. In this case one has to assume that the isomeric change occurs between two different molecules (instead of between parts of the same molecule) thereby linking them together.



In place of the second molecule of ester in the acetoacetic ester synthesis, a ketone may be substituted.

$$CH_3.COOC_2H_5 + CH_3.CO.CH_3 = CH_3.CO.CH_2.CO.CH_3 + C_2H_5.OH$$

Or, again, the first molecule of ester may be replaced by an aldehyde or ketone which then reacts in presence of an alkali

or organic base with the ester molecule. Benzaldehyde reacts with acetic ester, thus:

$$C_6H_5.CHO + CH_3.COOC_2H_5 = \underset{\text{Intermediate compound?}}{C_6H_5.CH(OH).CH_2.COOC_2H_5} \rightarrow \underset{\text{Cinnamic ester.}}{C_6H_5.CH{:}CH.COOC_2H_5}$$

In these cases water is removed and an unsaturated compound is obtained in the same way that crotonaldehyde is derived from aldol (p. 2). A similar reaction to the foregoing, known as *Perkin's reaction*, is effected between an aldehyde, the sodium salt of a fatty acid and its anhydride. Benzaldehyde, sodium acetate and acetic anhydride when heated together give cinnamic acid, the anhydride playing the part of a dehydrating agent.

$$C_6H_5.CHO + CH_3.COOH \rightarrow \underset{\text{Intermediate compound?}}{C_6H_5.CH(OH).CH_2.COOH} \rightarrow \underset{\text{Cinnamic acid.}}{C_6H_5.CH{:}CH.COOH} + H_2O$$

Although the intermediate aldol compound does not appear in the reaction, there can be little doubt that it is formed.

EXPT. 10.—**Preparation of Cinnamic acid.**—Mix in a small round flask furnished with a reflux condenser (250 c.c.) 20 grams of benzaldehyde, 10 grams of fused and powdered sodium acetate and 30 grams of acetic anhydride and heat the mixture to 180° in an oil-bath for about eight hours. The mass is poured out whilst hot into a large round flask (1 litre), sodium carbonate added until alkaline and any unchanged benzaldehyde distilled off in steam. After filtering from undissolved resinous by-products, hydrochloric acid is added, which precipitates the free cinnamic acid in colourless, crystalline flakes. It may be purified by recrystallisation from hot water. Yield 15–20 grams; m.p. 133°.

Carbon–Nitrogen chain formation.—The linking of carbon and nitrogen may be effected, as in the case of carbon and carbon, either by a process of substitution or addition. The formation of amines, for example, is brought about by the action of alkyl iodides on ammonia or amines (Vol. I, p. 204).

$$-NH_2 + CH_3I \rightarrow -NH.CH_3 + HI$$

Amides are formed by the action of ammonia and its substitution products on acids, acid chlorides and esters.

Oxalic ester and ammonia yield oxamide (Vol. I, p. 180).

$$\begin{matrix}COOC_2H_5\\ |\\ COOC_2H_5\end{matrix} + 2NH_3 = \begin{matrix}CO.NH_2\\ |\\ CO.NH_2\end{matrix} + 2C_2H_5.OH$$

In the same way diethyl malonic ester and urea in presence of sodium ethoxide give **veronal** (p. 132).

$$\begin{matrix}COOC_2H_5\\ |\\ (C_2H_5)_2C\\ |\\ COOC_2H_5\end{matrix} + \begin{matrix}NH_2\\ |\\ CO\\ |\\ NH_2\end{matrix} = \begin{matrix}CO-NH\\ |\quad |\\ (C_6H_5)_2C\quad CO\\ |\quad |\\ CO-NH\end{matrix} + 2C_2H_5.OH$$

Veronal.

Aldehydes and ketones react with hydrazines and hydroxylamine (Vol. I, p. 51). Acetoacetic ester and phenylhydrazine form a phenylhydrazone which, on gently heating, loses alcohol and passes into the ring compound, namely, phenylmethyl pyrazolone. When methylated it forms *antipyrine* (p. 135).

$$\begin{matrix}CH_3.CO\quad H_2N.NH.C_6H_5\\ |\\ CH_2-COOR\end{matrix} \rightarrow \begin{matrix}CH_3.C:N.NC_6H_5\\ |\quad |\\ H_2C-\!\!-CO\end{matrix}$$

Phenyl methyl pyrazolone.

EXPT. 11.—**Preparation of Phenyl methyl pyrazolone.** — Mix together 10 grams of dry phenylhydrazine hydrochloride (Vol. I, p. 276) and 9 grams of acetoacetic ester in a flask (200 c.c.), add three or four drops of conc. hydrochloric acid and warm for ten to fifteen minutes on the water-bath. A clear reddish solution is obtained which is poured into water and carefully neutralised with sodium hydroxide. The precipitated oil solidifies almost immediately and can be recrystallised from alcohol. Yield about 8 grams; m.p. 127°.

Unsaturated ketones unite with ammonia. Mesityl oxide (p. 14) and ammonia yield an additive compound, **diacetonamine**, which is used in the preparation of **β-eucaine** (p. 134).

$$\begin{matrix}CH_3\\ \diagdown\\ \quad C:CH.CO.CH_3\\ \diagup\\ CH_3\end{matrix} + NH_3 \rightarrow \begin{matrix}CH_3\\ \diagdown\\ \quad C(NH_2)\cdot CH_2.CO.CH_3\\ \diagup\\ CH_3\end{matrix}$$

Mesityl oxide. Diacetonamine.

EXPT. 12.—**Preparation of Diacetonamine.**—Fifty c.c. of mesityl oxide (*see* p. 14) are dissolved in twice their volume of spirit (64 o.p.), cooled in a freezing mixture to −10°, and dry ammonia is

passed into the solution until saturated. The solution is allowed to stand for two days. The base is separated as the acid oxalate. Mix 30 grams of powdered oxalic acid with 120 c.c. of spirit (64 o.p.) in a beaker placed in ice. Add the ammoniacal solution gradually so that the temperature does not rise above 20°, and stir vigorously until the pasty mass is neutral. To the resulting mixture, which is nearly solid, add a further 30 grams of powdered oxalic acid and stir until the mixture can be poured out. Pour it into a tin vessel fitted with reflux condenser and boil for fifteen minutes to complete the reaction. The product is filtered hot and the solution cooled slowly to 0°. The diacetonamine oxalate separates in crusts, which are filtered. The yield from 50 c.c. of mesityl oxide is about 50 grams of diacetonamine oxalate.

Note.—Diacetonamine can also be prepared directly by the action of ammonia on acetone.

Cyanamide, cyanic acid, and the carbimides unite with amino-compounds to form urea and guanidine derivatives, according to the following general equations:

$$\underset{\text{Amino-compound.}}{-NH_2} + \underset{\text{Carbimide.}}{CO.NR} \rightarrow \underset{\text{Urea derivative.}}{-NH.CO.NHR}$$

$$\underset{\text{Amino-compound.}}{-NH_2} + \underset{\text{Cyanamide.}}{CN.NH_2} \rightarrow \underset{\text{Guanidine derivative.}}{-NHC(:NH).NH_2}$$

Methyl glycine (**sarcosine**) combines with cyanamide to form **creatine** (p. 54).

$$\underset{\text{Sarcosine.}}{\begin{matrix}NH.CH_3\\|\\CH_2.COOH\end{matrix}} + CN.NH_2 = \underset{\text{Creatine.}}{\begin{matrix}&&NH\\CH_3.N-&C&\diagdown\\&|&NH_2\\&CH_2.COOH&\end{matrix}}$$

Substances known as **hydantoins** are obtained by the action of cyanic acid (potassium cyanate in presence of acid) on amino-acids. Hydantoin itself is derived from glycine.

$$\underset{\text{Glycine.}}{NH_2.CH_2.COOH} + HOCN = \underset{\text{Hydantoin.}}{\begin{matrix}NH.CH_2.CO\\|\quad\quad|\\CO\cdots\cdots NH\end{matrix}} + H_2O$$

The preparation of the hydantoins from glycine and tyrosine is described on pp. 74, 75.

Thiocarbimides behave like the carbimides and form thiocarbamides.

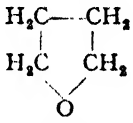
$$-NH_2 + CS.NR \rightarrow -NH\,CS.NHR.$$

Thiocarbamide.

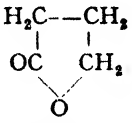
Carbon–Oxygen chain formation. — Chain formation between carbon and oxygen is represented by the ethers, esters and anhydrides. It is only in the former that the union can be regarded as stable.

CH_2-O-CH_2	$CO-O-CH_2$	$CO-O-CO$
Ether group (stable).	Ester group (less stable).	Anhydride group (least stable).

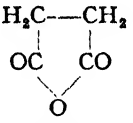
Such combinations may occur either in open chains, as represented by such substances as ethyl ether, ethyl acetate, and acetic anhydride, or by closed chains such as tetramethylene oxide, the inner ester or **lactone** of hydroxybutyric acid and succinic anhydride.



Tetramethylene oxide.



Hydroxybutyrolactone.



Succinic anhydride.

It should be remarked that the stability of a ring depends upon the number of atoms composing it, the most stable being those with 5 or 6 atoms. Is there any explanation of this? An ingenious and very plausible theory has been advanced by Baeyer under the name of the **Strain Theory.** It is based on stereochemical considerations, that is, upon the spatial distribution of the four carbon bonds. Supposing these bonds to be directed towards the solid angles of a regular tetrahedron they will make angles of 109°28′ with one another. Any distortion or deviation of these valency directions will lead, according to the theory, to a condition of strain, which will make itself evident by a loss of stability, and the greater the strain the greater the instability. Baeyer regards an olefine as the first member of the cyclic series, in which the normal position of the two bonds uniting the carbon atoms is assumed to form straight parallel links between the atoms.

The amount of distortion can be estimated, for each bond is bent inwards through half the total angle which the two make with one another, $\frac{1}{2}(109°28') = 54°44'$; in a cyclopropane derivative, in which the carbon atoms may be supposed to make an equilateral triangle, the amount of displacement will be $\frac{1}{2}(109°28'-60°) = 24°44'$. The amount of deviation from the normal is given in the following table:

Cycloethane (Ethylene)	$\frac{1}{2}(109°28')$	$54°44'$
Cyclopropane . . .	$\frac{1}{2}(109°28'-60°)$	$24°44'$
Cyclobutane . . .	$\frac{1}{2}(109°28'-90°)$	$9°44'$
Cyclopentane . . .	$\frac{1}{2}(109°28'-108°)$	$0°44'$
Cyclohexane . . .	$\frac{1}{2}(109°28'-120°)$	$-5°16'$
Cycloheptane . . .	$\frac{1}{2}(109°28'-128°34')$	$-9°33'$
Cyclo-octane . . .	$\frac{1}{2}(109°28'-135°)$	$-12°46'$

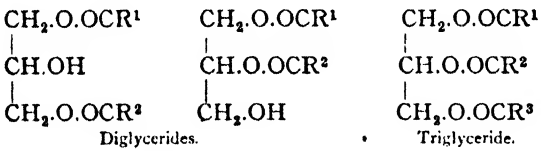
It will be seen that the condition of greatest strain will occur in the olefine, that of least strain in the cyclopentanes, and then in the cyclohexanes. In the last three the strain will be outwards instead of inwards. The same reasoning may be applied to other ring structures, and there are many facts which bear out this view.[1]

[1] *Organic Chemistry for Advanced Students*, by J. B. Cohen, Part I p. 178; E. Arnold, London, 1918.

CHAPTER II

THE OILS AND FATS

THE fats which occur in nature are glycerol esters or glycerides of the fatty acids (Vol. I, p. 118). When solid they are termed fats, when liquid, oils. These esters may contain the same or different fatty acid radicals; a diglyceride may possess two, a triglyceride three, different radicals (R = acid radical).



Diglycerides. Triglyceride.

Fats and oils, moreover, are not, as a rule, single chemical substances but mixtures of several esters, together with varying quantities of a substance termed **cholesterol** (p. 28). It follows therefore that the number existing among biochemical products is large.

Fats and oils are generally extracted by means of dry ether or petroleum ether in a Soxhlet extractor, *S*, shown in Fig. 2. In the case of oils and fats the material is first dried in a steam oven. Oil seeds are crushed before drying. The following example will illustrate the method.

EXPT. 13.—**Estimation of Butter-fat in Milk.**—Purify 200 c.c. of ether by allowing it to stand overnight with 20 grams of coarsely powdered potassium hydroxide and then distilling from the water-bath. Cut a strip of filter-paper, about 36 cm. (14 in.) long and 8 cm. (3 in.) wide, so that when loosely rolled up it will slip easily into the extractor and not project above the syphon tube, and pin it horizontally at two ends to wooden blocks.

Measure out 5 c.c. of fresh milk (stir it well beforehand) with a pipette and allow the milk to drop slowly from the pipette on to the paper in such a way that it spreads evenly over the surface. Warm the paper very cautiously from below with a small Bunsen flame so as to drive off the water. When dry, or nearly so, roll it up and tie it round with a thread, and leave in a steam oven for half an hour. Fit up an apparatus as shown in Fig. 2.

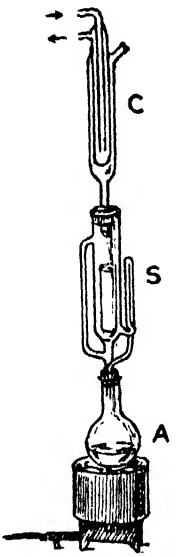


FIG. 2.

It consists of a clean and dry 200 c.c. flask, *A*, which is carefully weighed together with a small piece of broken porous pot and is then about half filled with purified ether. The flask is surmounted by a Soxhlet extractor to which a condenser, *C*, is attached. The whole is placed on the water-bath. The ether distils and the vapour passes by the side-tube (on the left of the figure) into the condenser, whence the condensed ether drips on to the filter paper filling up the extractor to the top of the syphon tube (on the right of the figure) from which it runs into the flask carrying with it the dissolved fat. After the syphoning process has been repeated six or seven times the flask is cooled and the ether removed by distillation from the water-bath. The flask is placed in a steam-oven for fifteen minutes, a little air blown over the surface to drive off the remaining ether and the flask weighed.

Example: Five c.c. of milk, when dried and extracted, gave the following weight of fat.

Flask + fat	41·043 grams
Empty flask	40·878 ,,
Weight of fat	0·165 gram

The weight of fat in 100 c.c. of milk = 0·165 × 20 = 3·3 grams.

The fatty acids which are present in oils and fats as glycerides belong to various groups, such as the saturated fatty acids (see Vol. I, p. 116), the unsaturated acids of the acrylic acid series, *e.g.*, oleic acid, $C_{18}H_{34}O_2$, found in fats and olive oil, etc., those with two pairs of double bonds, such as linoleic acid, $C_{18}H_{32}O_2$,

and with three pairs of double bonds represented by linolenic acid, $C_{18}H_{30}O_2$, obtained from linseed oil.

Further, there exist unsaturated hydroxy-acids such as ricinoleic acid, which occurs as the glyceride in castor oil.

The following table gives a list of the more important acids occurring as glycerides in natural fats and oils.

Acids of the Natural Fats and Oils.

Saturated Fatty Acids, $C_nH_{2n}O_2$.

Butyric	$C_4H_8O_2$	Lauric	$C_{12}H_{24}O_2$
Valeric	$C_5H_{10}O_2$	Myristic	$C_{14}H_{28}O_2$
Caproic	$C_6H_{12}O_2$	Palmitic	$C_{16}H_{32}O_2$
Caprylic	$C_8H_{16}O_2$	Stearic	$C_{18}H_{36}O_2$
Capric	$C_{10}H_{20}O_2$	Arachidic	$C_{20}H_{40}O_2$
		Behenic	$C_{22}H_{44}O_2$

Unsaturated Acids : Oleic series, $C_nH_{2n-2}O_2$.

Hypogaeic and Gaidic[1]	$C_{16}H_{30}O_2$
Oleic and Elaidic	$C_{18}H_{34}O_2$
Erucic and Brassidic	$C_{22}H_{42}O_2$

The hydrolysis of the fats and oils may be effected by alkalis, by acids and also by the enzyme, *lipase*, which is present in certain seeds (castor oil seeds) and also in the pancreatic juice and liver.

EXPT. 14.—**Preparation of Palmitic acid and Glycerol from Palm oil.**—Dissolve 17 grams of sodium hydroxide in its own weight of water and add it to 30 grams of palm oil previously melted in a large basin on the water-bath. Heat the mixture and stir for half an hour and add half a litre of boiling water, when a solution should be obtained. Add 75 c.c. of conc. hydrochloric acid gradually and continue to heat until the palmitic acid separates as a transparent brown oil on the surface. Cool and remove the cake of impure acid (keep the aqueous portion), press between filter paper and melt in a small basin on the water-bath when the oil may be decanted from the rest of the water. It is poured into a retort (250 c.c.) and distilled *in vacuo*. The neck of the

[1] The existence of isomeric pairs of unsaturated acids is explained on stereochemical grounds, for which the reader is referred to *Theoretical Organic Chemistry*, by J. B. Cohen, p. 363. Macmillan & Co., London, 1918.

retort is fixed into a small filter tube which serves as receiver as shown in Fig. 3.

A few small pieces of unglazed pot are dropped into the retort, the tubulus of which is closed with a cork holding a thermometer. Before commencing the distillation the apparatus should be tested to see that it is air-tight. It is then evacuated with the water-pump (see Vol. I, p. 277), and the distillation begun. It is advisable to hold the burner and to heat the retort with the bare flame. Under a pressure of 36 mm. the oil distils at about 245°. The flame should be moved about gently and the liquid kept boiling *vigorously*. The pale yellow oil which collects in the receiver is poured into a basin whilst hot and allowed to cool. It is spread on a porous plate and left to drain, when it becomes colourless, and after one or two crystallisations is pure. The yield is about 20 grams; m.p. 62°.

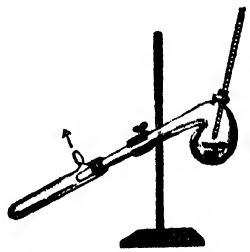


FIG. 3.

The aqueous portion from which the acid is originally removed contains free hydrochloric acid, sodium chloride and glycerol. The latter may be obtained by evaporating to dryness on the water-bath and extracting the residue with small quantities of alcohol. On evaporating the alcohol impure glycerol is left. It may be purified by distillation under reduced pressure, but the quantity is usually too small for the purpose.

Analysis of Fats and Oils.—The nature of these substances is ascertained from their physical constants, melting-point, refractive index, etc., and by certain chemical properties. The **saponification value** is the number of milligrams of potassium hydroxide required to hydrolyse 1 gram of the fat. The **iodine value** gives an estimate of the quantity of unsaturated acids present (for these substances like the olefines absorb iodine: Vol. I, p. 128) and is the amount of iodine in grams absorbed by 100 grams of the substance. In addition to these values it is important to ascertain the amount of volatile acid (such as butyric acid from butter) known as the **Reichert-Meissl value**, which gives the number of c.c. of $N/10$-potassium hydroxide solution required to neutralise the volatile fatty acids from 5 grams of fat, and the **acetyl value**, which, by forming an acetyl derivative,

determines the amount of hydroxy-acid present, as in the acid of castor oil.

EXPT. 15.—(*a*) **Saponification value.**—Dissolve about 20 grams of potassium hydroxide in about an equal weight of water in a 500 c.c. flask and make up to the mark with purified spirit (prepared by allowing ordinary spirit to stand for twenty-four hours over about 5 per cent. of coarsely powdered potassium hydroxide and then distilling). Titrate 25 c.c. of this solution (delivered from a pipette) against a normal or half-normal solution of hydrochloric acid using phenolphthalein as indicator. Weigh out 1 to 2 grams of lard into a ¼ litre flask, add 25 c.c. of the alcoholic potash solution and heat on the water-bath for half an hour with reflux condenser. Cool and titrate the excess of alkali.

Example.—1·1589 grams of lard, after saponification with 25 c.c. of alcoholic potash (25 c.c. = 15·9 c.c. *N*-HCl), require 11·7 c.c. of *N*-HCl for neutralisation.

$$\frac{(15{\cdot}9-11{\cdot}7)\times{\cdot}0561}{1{\cdot}1589} = 0{\cdot}197 \text{ KOH in combination with the}$$

fatty acids. The saponification value is, therefore, 197.

(*b*) **The Iodine value** is determined by estimating the amount of chloride of iodine absorbed by the oil or fat from a standard acetic acid solution of ICl, the absorption being due to the presence of unsaturated acid radicals (Vol. I, p. 192).

The number represents the weight in grams of iodine required to combine with 100 grams of the substance.

The solution is prepared by dissolving 13 grams of iodine in 1 litre of glacial acetic acid and passing through the solution a slow current of chlorine until the deep red colour changes to orange. This change, which takes place suddenly, is due to the formation of the monochloride and is known as *Wijs's solution.* A standard solution of sodium thiosulphate is prepared by dissolving about 24 grams of the solid in 1 litre of water and titrating against a standard iodine solution containing 10 grams in the litre, or, more simply, by using a solution of potassium dichromate containing 3·865 grams of salt in the litre, each c.c. of which liberates 0·01 gram of iodine from potassium iodide solution. Pour into a flask 10 c.c. of a 10 per cent. solution of potassium iodide and add 5 c.c. of dilute hydrochloric acid. Run in from a burette 20 c.c. of the dichromate solution (= 0·2 gram of iodine) and titrate with the thiosulphate solution until the iodine colour changes to straw, when a little thin starch solution is added and the end point ascertained by the disappearance of the blue

colour. Having ascertained the strength of the thiosulphate, weigh out 0·5 to 1 gram of lard into a ½ litre stoppered bottle, add 10 c.c. of carbon tetrachloride. When the lard is dissolved add 25 c.c. of the Wijs's solution from a pipette. The bottle is shaken, to mix the contents, and placed for half an hour in the dark. Meantime, the strength of the Wijs's solution is ascertained by running into a flask of about 250 c.c. capacity 10 c.c. of the solution, adding 10 c.c. of a 10 per cent. potassium iodide solution, diluting with 150 c.c. of water, and titrating with the standard thiosulphate solution as previously described. After the sample has stood in the dark for the requisite time, 15–20 c.c. of 10 per cent. potassium iodide solution are added. The mixture is well shaken, diluted with 400 c.c. of water and titrated with the standard thiosulphate solution, the bottle being well shaken meanwhile. *Example.*—0·5098 gram of lard was dissolved in 10 c.c. of carbon tetrachloride and 25 c.c. of Wijs's solution added (25 c.c. = 54·25 c.c. of thiosulphate solution, 6·6 c.c. of which were equivalent to 0·2 gram of iodine). The excess of iodine required 28·4 c.c. of thiosulphate solution. Hence the absorbed iodine is 54·25 − 28·4 = 25·8 c.c. of thiosulphate solution, and the amount of iodine per 100 grams of lard is given by the following expression :

$$\frac{0{\cdot}2 \times 25{\cdot}8 \times 100}{16{\cdot}6 \times 0{\cdot}5098} = 60{\cdot}9 \text{ grams.}$$

The iodine value of the lard is, therefore, 60·9.

(*c*) **The Reichert-Meissl value.**—Weigh out carefully 5 grams of melted butter into a 200 c.c. flask and add an alcoholic solution of sodium hydroxide (prepared by dissolving 2 grams of solid sodium hydroxide in 2 c.c. of water and adding 10 c.c. of pure alcohol). Heat the mixture with reflux condenser on the water-bath for fifteen minutes. Remove the condenser and boil off the alcohol on the water-bath. When the residue is dry, add 100 c.c. of distilled water, which has just been boiled to drive off carbon dioxide and heat till a solution is obtained. Acidify with 40 c.c. of normal sulphuric acid and fit the flask with a bulb adapter (as shown in Vol. I, Fig. 58, p. 239) and condenser, and distil 110 c.c. in the course of about half an hour. Cool the distillate, filter and titrate 100 c.c. with *N*/10-sodium hydroxide solution using phenolphthalein as indicator. The number of c.c.'s of *N*/10-alkali multiplied by 1·1 is the Reichert-Meissl (or Reichert-Wollny) value (the multiplier 1·1 gives the correction for the 110 c.c. of distillate).

Example.—Five grams of butter required 25·2 c.c. of *N*/10-

NaOH solution, $25{\cdot}2 \times 1{\cdot}1 = 27{\cdot}7$, which is the Reichert-Meissl value.

The following table gives the constants for various fats and oils.

Oil or fat.	Saponification value.	Iodine value.	Reichert-Meissl value.
Palm-nut . . .	242–250	13–17	5–6·8
Cocoa-nut . . .	246–260	8–10	6·6–7·0
Linseed D.[1] . .	192–195	173–201	0·0
Cotton-seed S.D.[1]	193–195	108–110	—
Olive N.D[1]. . .	185–196	79–88	0·6
Lard	195	76–85	0·68
Tallow (mutton) .	192–195	35–46	0·5
Goose fat . . .	193	59–71	0·2–0·3
Butter fat . . .	220–233	26–50	26–33

The Waxes.—In addition to the fats, there are found in the animal and vegetable kingdom esters of the higher alcohols consisting of colourless solids, which, in the compact form, are translucent and are known chemically as **waxes.** The following are the better known waxes and their source, together with the formula of the alcohol.

Alcohol.	Ester.	Source.
Cetyl alcohol $C_{16}H_{33}(OH)$	Cetyl palmitate	Spermaceti
Ceryl alcohol $C_{26}H_{53}(OH)$	Ceryl palmitate	Poppy wax
,,	Ceryl cerotate	Chinese wax
Myricyl alcohol $C_{30}H_{61}(OH)$	Myricyl palmitate	Bees wax
,,	Myricyl cerotate	Carnauba wax

Cholesterol, $C_{27}H_{45}OH$, was discovered in gall-stones, but it is very widely distributed in both the animal and vegetable organism. It is associated in small quantities with the fats and

[1] The initials D., S.D., and N.D. stand for "drying," "semi-drying," and "non-drying." The term has reference to the hardening of the oil by oxidation and is due to its unsaturated character. This is manifested mainly in the high iodine value, which is greatest in the drying oils

oils, is present in egg-yolk, bile, brain, blood, and in the liver, kidney and epidermis. It is also found in cod-liver oil. It crystallises from chloroform in needles which melt at 148–151°. It is soluble in most organic solvents, but not in water, and is consequently extracted with the fats and oils by ether.

EXPT. 16.—**Preparation of Cholesterol from Gall-stones.**—Powder a few grams of gall-stones, bring them on to a filter and wash them several times with hot water. Extract the residue with ten times its weight of alcohol by heating on the water-bath with a reflux condenser until only a small coloured residue remains undissolved. Filter quickly through a wide Buchner funnel to prevent the substance crystallising in the funnel. On cooling, lustrous, white laminæ separate and are filtered and dried; m.p. 148°. The yield varies and may reach 90 per cent. of the gall-stones.

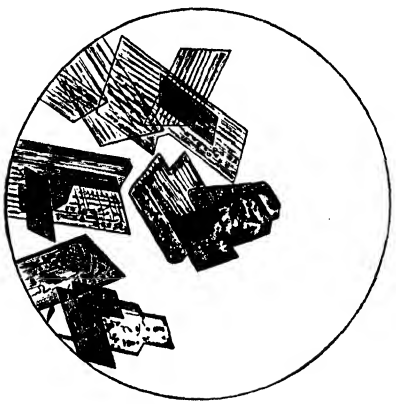


FIG. 4.—Cholesterol crystals (after Funke).

If a minute quantity of cholesterol is dissolved in chloroform and strong sulphuric acid added, the chloroform is coloured crimson which changes to purple; the sulphuric acid at the same time has a green fluorescence. A drop of the purple chloroform solution exposed to the air changes to blue, green and ultimately yellow. Another reaction is to dissolve the substance in acetic anhydride when, on adding strong sulphuric drop by drop, a violet pink colour is produced.

The constitution of cholesterol is still under investigation. It is a secondary alcohol, for it gives a ketone, *cholestenone*, on oxidation, and is almost certainly a cyclic compound containing a double bond.

Formation and decomposition of Fats in the Body.—As the fats and oils found in the animal body are largely derived

unaltered from the food, the biochemical synthesis is mainly restricted to the plant, about which, however, little is known. Nevertheless, there is enough evidence to show that animals are also capable of building up these substances from carbohydrates. It may be that the carbohydrates also furnish the materials for the plant; for unripe seeds contain carbohydrates which, on ripening, give place to oils and fats.

As in the process of alcoholic fermentation (see p. 105), it seems not improbable that the first stage in the process of animal synthesis is the disintegration of the hexose molecule into two groups of three carbon atoms, either as methyl glyoxal, $CH_3.CO.CHO$, or as lactic acid, $CH_3.CH(OH).COOH$ (p. 106).

If we suppose further that the latter yields acetaldehyde (as it is known to do with sulphuric acid),

$$CH_3.CH(OH).COOH \rightarrow CH_3.CHO + H.COOH$$

the aldol condensation (p. 13) may set in and produce chains of 4, 6, 8, etc., carbon atoms which by partial reduction and oxidation give fatty acids. It is well known that lactic acid can be transformed into butyric, capric and caproic acids by the action of the butyric ferment.

In this connection it is interesting to note that among the higher fatty acids those that occur in nature contain an even number of carbon atoms.

If, then, this process occurs in the case of carbohydrates, it may be applied also to the amino-acids of protein hydrolysis (p. 70), such as alanine, which may undergo oxidation to acetaldehyde by the intervention of some oxidising ferment (p. 103).

$$CH_3.CH(NH_2).COOH + O = CH_3.CHO + NH_3 + CO_2$$

The fats and oils are utilised as reserve materials for the plant and animal, and in the latter may sustain life for a considerable period. In what manner they are utilised is as yet little understood. Before they can pass through the cell-wall they must be hydrolysed, and this may take place by the aid of the fat-splitting enzyme, **lipase** (p. 103), which is known to be present in the intestinal wall. If this occurs there is evidence that the fat is re-formed, and the problem of its utilisation as a source

of energy is therefore not advanced. According to Leathes the fat is first transferred to the liver previous to oxidation, and is there converted into oxidisable glycerides of unsaturated acids; but the subsequent changes are still obscure. It is known that many acids with an even number of carbon atoms undergo oxidation to the β-ketonic acids, and they may furnish the initial stage for further disintegration. Butyric acid, for example, is known to yield aceto-acetic acid.

$$\overset{\gamma}{C}H_3.\overset{\beta}{C}H_2.\overset{\alpha}{C}H_2.COOH \rightarrow \overset{\gamma}{C}H_3.\overset{\beta}{C}O.\overset{\alpha}{C}H_2.COOH$$

Butyric acid. Acetoacetic acid.

To quote Prof. Leathes[1] "To sum up then, by piecing together what is probable with what is known of the chemistry of the processes by which the energy of fat molecules is rendered available in the animal body: the fat is transported to the liver, unsaturated unions are there introduced into the fatty acids and possibly there, too, the complex compounds of fatty acids with phosphorus and nitrogen built up.[2] The unsaturated products, which are next found in the cells of other organs throughout the body, break down, probably where the unsaturated links have been introduced, and the lower acids so formed by successive oxidation at the β-carbon atom, break down further to molecules of the size of acetic acid, which are lastly completely burnt to carbonic acid and water."

[1] *The Fats*, by J. B. Leathes, p. 115. Monographs on Biochemistry. Longmans, 1910.

[2] See p. 51.

CHAPTER III

THE CARBOHYDRATES

The Carbohydrates.—In Vol. I, p. 147, the occurrence and properties of the natural carbohydrates were discussed. In the present chapter it is proposed to describe their structure and synthesis so far as our present knowledge extends.

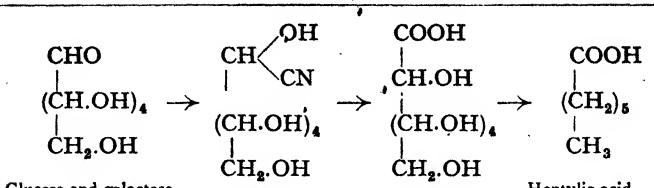
Structure of the Monosaccharoses.—Glucose, mannose, and galactose, the three natural hexoses, possess all the properties of aldehydes, combining, with hydrogen cyanide, hydroxylamine, phenylhydrazone (Vol. I, p. 150), and yielding on oxidation monobasic acids containing the original number of carbon atoms. They form penta-acetyl derivatives, and therefore contain five hydroxyl groups. On reduction they take up two atoms of hydrogen and form hexahydric alcohols, which with hydriodic acid are converted into normal, secondary hexyl iodide.

$$CH_3.CH_2.CH_2.CH_2.CHI.CH_3$$

Consequently, the three sugars consist of a normal chain of six carbon atoms. Five of these are probably present as carbinol groups, since it is unlikely that two hydroxyl groups are attached to one carbon atom, and the sixth will represent an aldehyde group.

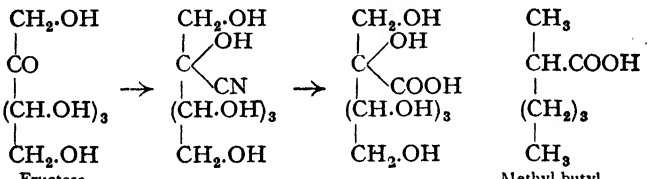
$$CH_2(OH).CH(OH).CH(OH).CH(OH).CH(OH).CHO$$

The existence of a normal chain in the case of glucose and galactose was further confirmed by Kiliani, who transformed these sugars into normal heptylic acid in the following way. The sugar is first converted into the cyanhydrin, hydrolysed to the acid and then reduced with hydriodic acid.



Glucose and galactose. Heptylic acid.

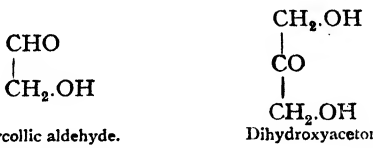
Although fructose possesses some of the reducing properties of glucose, and gives, on reduction, a hexahydric alcohol, it breaks up on oxidation into trihydroxybutyric acid and other products. Treated according to Kiliani's method it yields methyl butyl acetic acid as follows :



Fructose. Methyl butyl acetic acid.

Fructose is therefore a keto-hexose.

The elucidation of the structure of these sugars led to the synthesis of a variety of alcohol-aldehydes and -ketones containing from two (biose) to ten (decose) carbon atoms, all of which were found to possess the general characteristics of the monosaccharoses. The simplest of the aldoses is glycollic aldehyde (biose) ; that of the ketoses is dihydroxyacetone :



Glycollic aldehyde. Dihydroxyacetone.

The following synthetic methods have been used in their preparation :

1. The lower members by the action of an alkali undergo the aldol condensation. Glycollic aldehyde yields erythrose and glycerose [1] is converted into fructose.

[1] The term glycerose is given to the oxidation product of glycerol and consists of glyceric aldehyde and dihydroxyacetone.

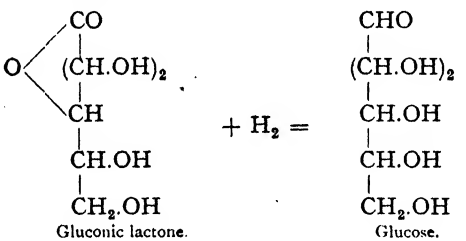
$$CH_2.OH.CHO + CH_2.OH.CHO = CH_2(OH).CH(OH).CH(OH).CHO$$
Erythrose.

$$CH_2(OH).\underbrace{CH(OH).CHO}_{\text{Glycerose.}} + CH_2(OH).CO.CH_2(OH) =$$
$$CH_2(OH).CH(OH).CH(OH).CH(OH).CO.CH_2(OH)$$
Fructose.

2. Polyhydric alcohols, on oxidation, give the corresponding aldose; mannitol (the hexahydric alcohol found in the manna ash) has been converted into mannose.

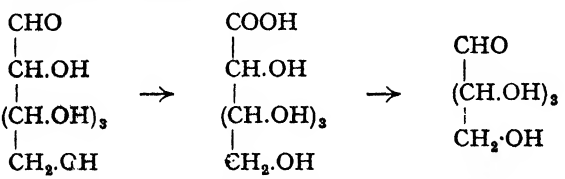
$$\underset{\text{Mannitol.}}{CH_2.OH(CH.OH)_4CH_2.OH} + O = \underset{\text{Mannose.}}{CH_2.OH(CH.OH)_4CHO} + H_2O$$

3. Lactones (p. 20) of hydroxy-acids may be reduced in faintly acid solution by means of sodium amalgam to aldehydes. Gluconic lactone gives glucose.



Gluconic lactone. Glucose.

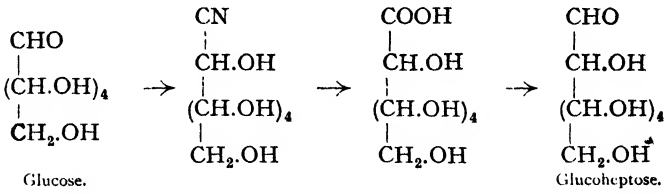
4. There are several methods by which an aldehyde group is removed from an aldose, and a lower monosaccharose obtained. One method is to oxidise the sugar to the corresponding acid and then further oxidise this with Fenton's reagent—hydrogen peroxide and a trace of ferrous salt—to the lower sugar. Glucose has been converted into arabinose.



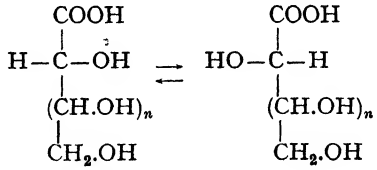
Glucose. Gluconic acid. Arabinose.

5. The reverse process, that of converting a lower into a higher aldose, may be effected through the cyanhydrin which is hydro-

lysed and the lactone reduced (see p. 20). Glucoheptose has been prepared by this method from glucose.

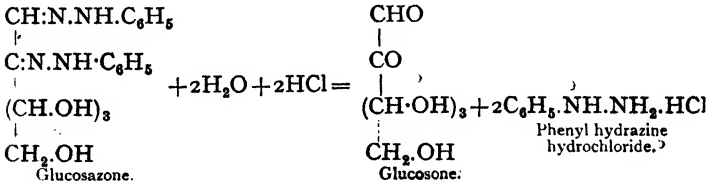


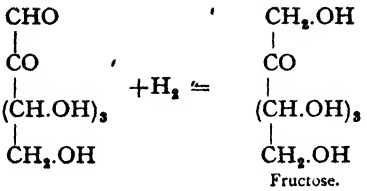
6. The interconversion of isomeric aldoses has been effected in the following way: the monobasic acids (derived from the sugars by oxidation) when heated in aqueous solution with pyridine to 130–150° undergo a molecular change, whereby the hydroxyl and hydrogen of the carbinol group next the carboxyl group are partially or completely interchanged. The reaction is analogous to the conversion of tartaric acid into racemic and mesotartaric acids (Vol. I, p. 187).



The new acid, thus obtained, may be reduced to the aldehyde.

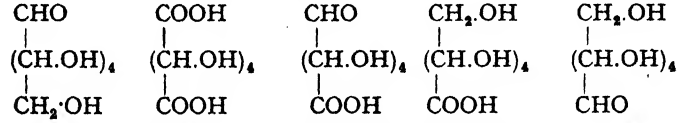
7. The conversion of an aldose into a ketose is effected through the osazone (Vol. I, p. 152). Glucosazone has been transformed into fructose. The glucosazone is first hydrolysed to glucosone and the latter on reduction gives fructose.





Fructose.

8. The interconversion of stereoisomeric aldoses has been carried out in the case of glucose by a process of graduated oxidation and reduction in such a way that the position of the end groups (carbinol and aldehyde) are reversed. The importance of this method will be better understood when the stereoisomerism of the monosaccharoses is considered.



Glucose. Saccharic acid. Glycuronic acid Gulonic acid. Gulose.

Glucose, on oxidation, passes first into the monobasic gulonic acid and, by further oxidation, into the dibasic saccharic acid. On reducing the latter, the reducing agent attacks the carboxyl representing the original aldehyde group, which is converted into the carbinol group.

EXPT. 17.—(*a*) **Estimation of Glucose and Invert-sugar with Fehling's solution.**—The Fehling solution is first prepared by making two solutions, one by dissolving 69·28 grams of pure, crystallised copper sulphate in 1 litre of water and the other by dissolving 350 grams of Rochelle salt (sodium potassium tartrate) and 100 grams of sodium hydroxide in 1 litre of water. When equal volumes of each of the above solutions are mixed the resulting liquid is known as *Fehling's solution* and is reduced to cuprous oxide by glucose. If 5 c.c. of each solution are taken, the 10 c.c. of the Fehling are exactly reduced by 0·05 gram of glucose. The process is carried out as follows. The sugar solution to be examined is introduced into a burette and a mixture of 5 c.c. of the two solutions placed in a porcelain basin to which 40 c.c. of water are added, and boiled gently over a small flame. The sugar solution is added to the hot liquid 1 to 2 c.c. at a time until (the precipitate of cuprous oxide being allowed to subside) the blue colour just vanishes. The process is repeated adding the sugar

solution more slowly towards the end (one drop at a time) in order to determine the exact point at which the precipitation is complete and no excess of sugar is present. Before testing a solution of glucose of unknown strength it is advisable to carry out an experiment with pure glucose or invert-sugar (see below) by making a solution of such a strength that 10 c.c. contains exactly 0·05 gram of the substance.

In case the preliminary examination of the solution to be tested requires a much larger or smaller volume than 10 c.c. to precipitate exactly the Fehling solution, the original sugar solution must either be concentrated or diluted so that approximately 10 c.c. of sugar solution = 10 c.c. of the Fehling solution.

As the end-point of the reaction is difficult to determine owing to the presence of a precipitate, *Ling's indicator* may be used. It consists of ferrous thiocyanate, which in presence of cupric sulphate is oxidised to red ferric thiocyanate. It is prepared by dissolving 1·5 grams of ammonium thiocyanate and 1·0 gram of ferrous ammonium sulphate in 10 c.c. of water at about 40°, cooling and then adding 5 c.c. of conc. hydrochloric acid. If the solution has a reddish colour a little zinc dust will remove it. The testing of the end-point is made by means of the drop method, that is, by placing a series of drops of the indicator on a white tile and touching the drops in succession with a drop of the solution. The reduction is complete when no red colour is produced by the Fehling solution.

Example.—The solution of **invert-sugar** was prepared as follows: 11·320 grams of cane-sugar were dissolved in water and made up to 500 c.c. Fifty c.c. of this solution were mixed with 10 c.c. conc. hydrochloric acid in 50 c.c. of water and warmed to 65–70° for fifteen minutes, and then made up to 250 c.c.

10 c.c. Fehling solution = 10·5 c.c. of sugar solution
= 0·0475 gram of cane-sugar.

$$\frac{0{\cdot}0475 \times 250}{10{\cdot}5} = 1{\cdot}131 \text{ grams of cane-sugar.}$$

EXPT. 18.—(*b*) **Estimation of Glucose by Benedict's solution.**—Benedict's solution is prepared by dissolving 200 grams crystallised sodium carbonate, 200 grams sodium citrate and 125 grams of potassium thiocyanate in 800 c.c. of hot water, filtering and cooling. Copper sulphate (18 grams) is dissolved in about 100 c.c. of water and added with constant shaking to the first solution. To this 5 c.c. of 5 per cent. solution of potassium ferrocyanide is added, and the total volume made up to a litre.

25 c.c. Benedict's solution = 0·05 gram of glucose.

The solution is standardised against a solution of pure glucose or invert-sugar of about 0·5 per cent. strength.

Sodium citrate replaces the less stable Rochelle salt of Fehling solution, potassium thiocyanate precipitates cuprous thiocyanate, whilst potassium ferrocyanide prevents the precipitation of cuprous oxide. The solution is stable, does not deteriorate on exposure to light and a small amount of protein does not affect the estimation.

Twenty-five c.c. of Benedict's solution and 3 grams of anhydrous sodium carbonate are placed in a small flask, which is fixed in a clamp and the contents slowly brought to the boil over a small flame, a few pieces of porous pot being added to prevent bumping. The sugar solution, which should be of about 5 per cent. strength, is run in from a burette as rapidly as possible without interrupting the boiling. As soon as the white precipitate of CuCNS begins to appear, the sugar solution is added more slowly, thirty seconds being allowed to elapse between each addition. The end point is indicated by the disappearance of the blue colour of the copper.

Example.—Twenty-five c.c. of Benedict's solution required 10·5 c.c. of invert-sugar solution (= 0·0475 gram of cane-sugar).

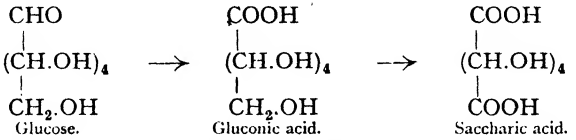
$$\frac{0{\cdot}0475 \times 250}{10{\cdot}5} = 1{\cdot}131 \text{ grams of cane-sugar.}$$

When the aldehyde sugars are oxidised, they yield first monobasic acids by conversion of the aldehyde to a carboxyl group, and secondly dibasic acids by the oxidation of the end carbinol group to carboxyl. Glucose yields in this way saccharic acid.

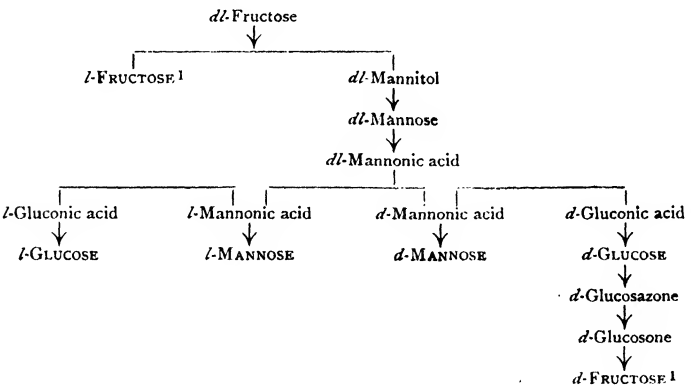
HOOC.CHOH.CHOH.CHOH.CHOH.COOH

EXPT. 19.—**Preparation of Saccharic acid from Glucose.**—Heat in a basin on the water-bath, with constant stirring, 50 grams of anhydrous glucose with 350 grams of nitric acid (sp. gr. 1·15) until a syrupy residue remains. Dissolve it in a little water and again evaporate. The operation is stopped when the mass begins to turn brown. Dissolve it in 150 c.c. of water and neutralise the solution with a concentrated solution of potassium carbonate; add 25 c.c. of 50 per cent. acetic acid and concentrate to about 80 c.c. On standing in the cold or by rubbing, the acid potassium salt crystallises. Let stand for twelve hours, filter at the pump, wash with a little cold water and recrystallise from the least possible quantity of hot water with the addition of animal

charcoal. The salt so obtained is colourless and should be free from oxalic acid. The yield is about 15 grams (Fischer).



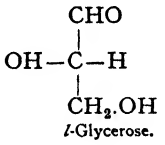
Synthesis of the Natural Monosaccharoses.—The starting point for the synthesis of the natural hexoses is fructose, which, as already explained, is obtained by the aldol condensation of glycerose. This substance, which, like most synthetic compounds, is a mixture of the two optical varieties (dextro and lævo), can be fermented by yeast, which removes only the natural fructose, leaving the dextrorotatory isomer. If the inactive fructose is first reduced it gives inactive mannitol, which may be oxidised to mannonic acid. The acid can be resolved by fractional crystallisation of the strychnine or morphine salt into its optical (*d* and *l*) components. Each of these by heating with water and pyridine is transformed into *d*- and *l*-gluconic acids, which on reduction yield the corresponding glucoses. Moreover, natural glucose, as we have seen (p. 35), has been converted into natural fructose. The following table represents the above changes.



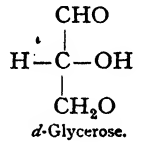
[1] The terms *d* and *l* applied to fructose have reference to their relation to glucose, but not to their rotations, which are actually the reverse of those represented by the symbols.

Configuration of the Monosaccharoses.—The term configuration has reference to the space or stereochemical arrangement of the carbon groups. In order to understand these relationships the student is referred to the subject of **optical activity** in Vol. I, p. 171. It was there pointed out that optical activity is correlated to the presence of an asymmetric carbon atom, that is, one to which four different atoms or atomic groups are attached. Such an asymmetric space arrangement can exist in two non-superposable forms, corresponding with an object and its mirror image or to a left and right hand. In lactic and malic acid only one asymmetric carbon atom is present, and only two space- or stereo-isomers can exist. With an increase in the number of such carbon atoms, the number of stereoisomers will increase according to the general formula 2^n, in which n stands for the number of asymmetric carbon atoms.

The lowest member of the aldoses which contains an asymmetric carbon atom is glyceric aldehyde, which can consequently exist in two stereoisomeric forms, both of which have been prepared.



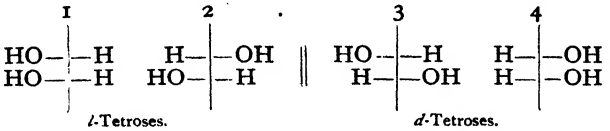
l-Glycerose.



d-Glycerose.

Assuming the left-hand configuration (which must be regarded as a space arrangement) to represent the lævo-compound, then the right-hand configuration will be the dextro-compound. On this basis we may proceed to build up the other aldoses by adding new carbinol groups, each containing a new asymmetric carbon atom above that already present. In order to simplify the formula, the asymmetric carbon may be indicated by cross lines and the aldehyde and primary alcohol group, which maintain the same positions, eliminated from the top and bottom of the formula.

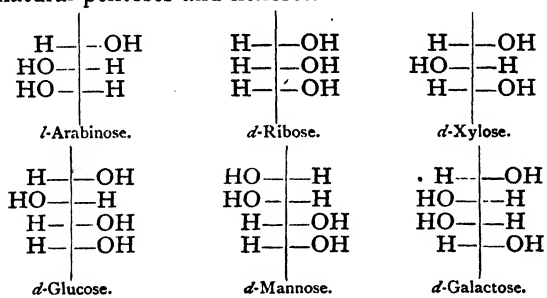
The two glyceroses (trioses) should then give rise to four tetroses, which may be represented by adding a hydroxyl group and a hydrogen atom alternately to one or other side of the cross lines.



l-Tetroses. *d*-Tetroses.

Each of these in turn will yield two pentoses making 8 stereoisomeric pentoses, and each pentose two hexoses making 16 stereoisomeric hexoses. All the aldo-pentoses have been synthesised and 14 out of the 16 aldo-hexoses are known.

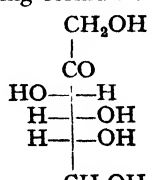
It is beyond the scope of the present volume to discuss in detail the method by which the configuration of these various stereoisomers has been determined; but it is not difficult to understand that, assuming the above configuration of the two glyceroses, it might be possible by building up the more complex aldoses or breaking down the latter into simpler sugars to establish stereoisomeric relations between them. The actual process is described in special treatises dealing with the carbohydrates. It must suffice here to give the names and configurations of the natural pentoses and hexoses.



l-Arabinose. *d*-Ribose. *d*-Xylose.

d-Glucose. *d*-Mannose. *d*-Galactose.

d-Fructose (natural fructose), as it gives the same osazone as *d*-glucose (Vol. I, p. 153), must contain three asymmetric carbinol groups having the same configuration as the latter, and will therefore have the following formula:



d-Fructose.

Fischer has made the interesting observation that only sugars containing 3 (or a multiple of 3) carbon atoms undergo fermentation by yeast, and of all the hexoses only the four natural substances are attacked. This remarkable specific or selective action on the part of the yeast-cell has been compared by Fischer to a lock and key. The active agent of the yeast is a protein substance which is optically active and therefore asymmetric in structure. If the atomic grouping of the protein molecule fits that of the sugar molecule as the wards of a key fit the lock, fermentation occurs; otherwise there is no action. It is a significant fact that the configuration of glucose, mannose and fructose is the same in respect of the three lower asymmetric carbinol groups; but differs by two in galactose, which, it may be added, ferments less readily than the other three sugars.

The Alkyl glucosides.—The term alkyl glucoside has been given by Fischer to compounds of the sugars with the alcohols. Methyl glucoside is obtained by the action of hydrochloric acid upon a mixture of glucose and methyl alcohol.

$$C_6H_{12}O_6 + CH_3.OH = C_6H_{11}O_5.OCH_3 + H_2O$$

As the new compound has lost its aldehyde properties it is represented by the following formula:

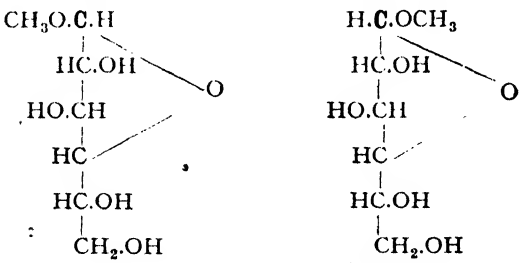
$$CH_2.OH.CH.OH.CH.CH.OH.CH.OH.CH.OCH_3$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad O$$

EXPT. 20.—**Preparation of α-Methyl glucoside.**—Dehydrate methyl alcohol (free from acetone) [1] by leaving it over quicklime for several days. Place 10 c.c. in a flask, which is weighed together with a delivery tube, well cooled, so that no evaporation takes place, and dry hydrogen chloride passed in for a short time. The increase in weight is ascertained and the methyl alcohol diluted with the same solvent until it contains 0·25 per cent. of hydrogen chloride. To 100 grams of this dilute solution, add 25 grams of anhydrous and finely powdered grape-sugar and boil with reflux condenser for three-quarters of an hour until the sugar is completely dissolved. The pale yellow liquid probably contains

[1] A simple way of removing acetone from commercial methyl alcohol is to pass a current of chlorine through the boiling liquid until saturated, letting it stand over quicklime to remove free chlorine and then fractionating with a column. The acetone is converted into a high boiling chloracetone which remains in the distilling flask.

glucosedimethylacetal, which is converted into the glucoside on heating to 100°. Place the solution in a sealed tube and heat for fifty hours in boiling water. The solution is then evaporated to one-third of its volume and well cooled. The α-methyl glucoside slowly deposits in small, colourless needles, which, after standing twelve hours, are filtered. The yield is about 45 per cent. of the grape-sugar. It is purified by crystallisation from eighteen parts of ethyl alcohol. (Fischer.)

If this is the correct explanation, the formation of the methyl glucoside must be accompanied by the creation of a new asymmetric carbon atom (indicated in the formula in thick type) and consequently of two isomers. This is precisely what occurs, and with the majority of aldoses two stereoisomers, distinguished as α and β, have been isolated. This structure of the α- and β-methyl glucosides may be represented in the following manner:



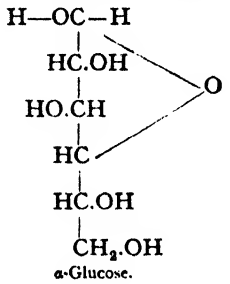
α- and β-Methyl glucosides.

Fischer made the interesting observation that an enzyme extracted from pulverised yeast-cells or **maltase** (see p. 101) hydrolyses α-methyl glucoside into glucose and methyl alcohol, but has no action on the β-compound, whereas **emulsin**, an enzyme present in bitter almonds, has the reverse effect.

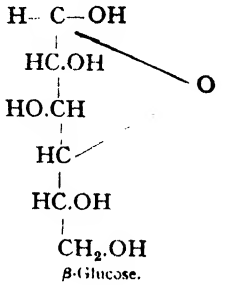
This selective action of the two enzymes is found to apply to the natural glucosides such as amygdalin, salicin, etc., which are treated in a subsequent section (p. 102). The majority are hydrolysed by emulsin and are therefore β-glucosides.

Now glucose itself appears to exist in isomeric forms, for a fresh solution has a specific dextrorotation of $[a] = +110°$, which falls on standing, or, more rapidly on the addition of alkali, to $+52{\cdot}5°$. Glucose crystallised from alcohol, when freshly dissolved, has a rotation of $[\alpha]_D = +20°$, which also changes, becoming

constant at + 52·5°. The explanation, which is supported by experiment, is that glucose itself exists in α- and β-forms having respectively the rotation + 110° and + 20° and that the sugar of rotation 52·5° is an equilibrium mixture of the two. These substances are therefore not aldehydes but lactones which pass by tautomeric change (p. 15) into aldehydes.

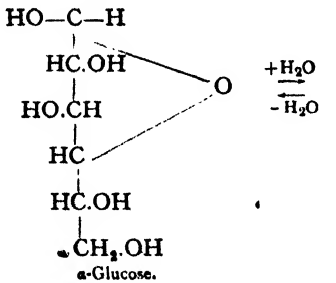


α-Glucose.

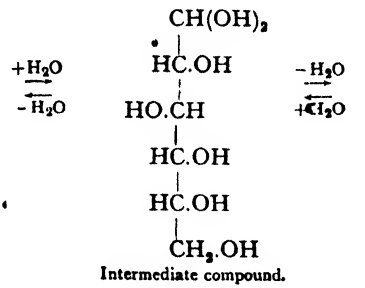


β-Glucose.

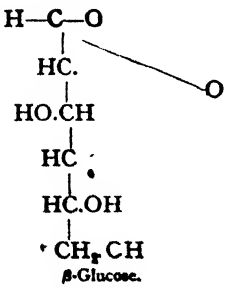
The manner of their interconversion in the formation of the mixture is represented by the addition and loss of the elements of water, thus :



α-Glucose.



Intermediate compound.

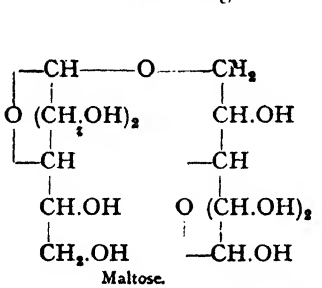


β-Glucose.

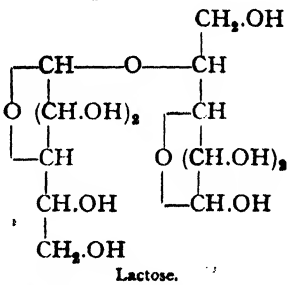
This action of the two enzymes has been applied to the disaccharoses, which can, in the same way, be divided into α- and β-compounds, the one group being resolved by maltase and the other by emulsin.

Thus maltose is a glucose α-glucoside, whereas lactose is a glucose β-galactoside.

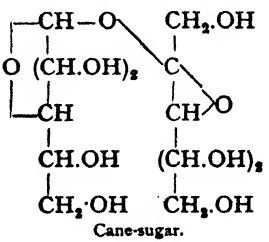
The Disaccharoses.—Among the many natural di-, tri- and tetra-saccharoses which recent investigation has brought to light, the most familiar are the **disaccharoses**, cane-sugar (sucrose), milk-sugar (lactose) and malt-sugar (maltose). Each is hydrolysed by acids and breaks up into two molecules of hexose as already explained (Vol I, pp. 149, 153, 161); but whereas cane-sugar has no reducing action and forms no osazone, the two latter behave like monosaccharoses, being reducing sugars and forming well-defined osazones. It would naturally follow that cane-sugar possesses no aldehyde or ketone group whilst lactose and maltose do. For this and for other reasons the following formulæ have been assigned to these three sugars.



Maltose.



Lactose.



Cane-sugar.

Expt. 21.—Analysis of Cane-sugar by the Polarimeter.—The rotatory power of an optically active substance (see Vol. I,

p. 171) is determined by means of a polarimeter. One of these instruments is shown in Figs. 5 and 6.

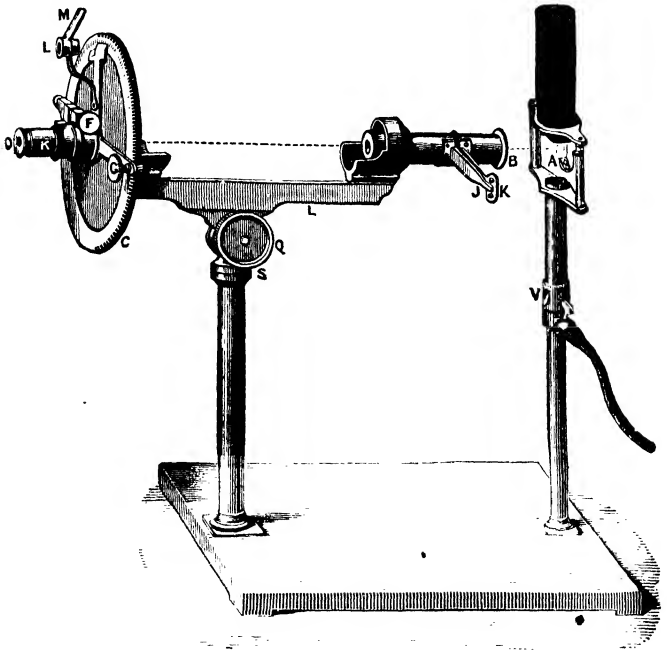


FIG. 5.

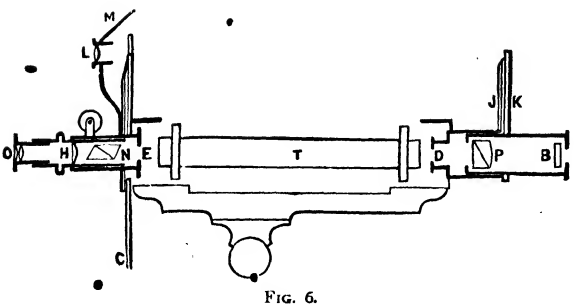


FIG. 6.

The monochromatic light of a sodium flame is commonly used in these determinations and is obtained by suspending in the flame of a Mekker, or other form of burner, a platinum wire

basket containing fused sodium chloride. The light from the flame passes through a cell *B*, containing a solution or crystal of potassium dichromate, which deprives it of blue or violet rays. It then passes through the polarising Nicol prism, *P*. A plate of quartz cut parallel to the optic axis covers half the opening of *D*, and is of such a thickness that it produces a difference of a half-wave length (or an exact odd multiple of a half-wave length) between the two rays, which it gives by double refraction. The light then passes through the liquid placed in the tube *T*, and entering at *E* strikes the analysing Nicol, *N*. The telescope, *O H*, is focussed on the edge of the quartz-plate at *D*. When *N* is turned, a pointer moves over the graduated circle, *C*, and its position can be read by means of the lens, *L*.

The theory of the instrument may be explained as follows:

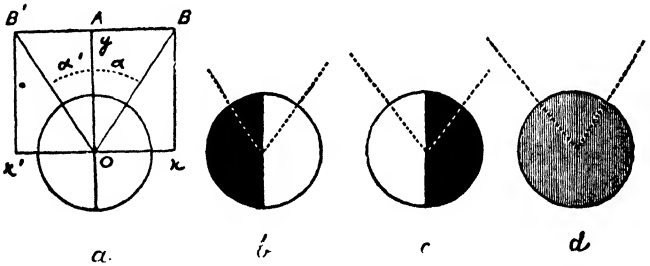


FIG. 7.

If, after passing through the Nicol *P*, the plane of vibration is in the direction *OB*, Fig. 7*a*, then in the half of the field to the right, uncovered by the quartz plate, it passes on unchanged. When it strikes the quartz the ray is broken up into the two components *Oy* and *Ox*. These traverse the quartz with different velocities, and since one ray is retarded half a wave-length in respect of the other, the vibration of one component will be represented by *Oy*, but the other must be represented by *Ox'* instead of *Ox*. These two combine on emerging to a plane polarised ray vibrating in the direction *OB'* so that the angle *A OB'* is equal to the angle *A OB*. If now (the tube containing water or other non-rotating liquid) the Nicol *N* be so placed that it is parallel to Nicol *P*, then the light in the half of the field to the right will pass through unchanged, but only a portion of the light which has passed through the quartz diaphragm with its plane of vibration in the direction *OB'* will pass through *N*, and consequently there will be different intensities of illumination in the two halves of the field, Fig. 7*b* (if the angle *a* is 45° then

the angle BOB will be 90°, and the light in the left half of the field will be completely obscured). Similarly, if the plane of the Nicol N be made parallel to OB', there will be a greater intensity of illumination in the left half of the field, Fig. 7*c*. Between the two positions of the Nicol N there must necessarily be one which gives uniform illumination of the whole field, and this is the zero point of the instrument, Fig. 7*d*.

If the tube, T, containing the active substance, be interposed between the two Nicols, then both rays, OB and OB', will be rotated through equal angles, and to re-establish uniform illumination in the two halves of the field the Nicol N must be turned through an angle equal to the angle of rotation, which is then measured on the divided circle. When the angle α is small, *i.e.*, when the plane of vibration of the polarised light is almost parallel to the optic axis of the quartz, the greatest degree of sensitiveness is attained, for then a very small change in the position of N causes a great difference in the respective illuminations in the two halves of the field. As α increases, the sensitiveness diminishes, but a greater total intensity of illumination is obtained. By moving J (Fig. 6) the position of the Nicol P may be altered. For clear, colourless liquids the angle α may be made comparatively small; but in the case of coloured liquids it is necessary to have α larger, and so obtain a greater intensity of light at the cost of sensitiveness.

The angle of rotation, represented by α_D (for sodium light), varies with the length of the column of liquid through which the light passes. One decimetre has been chosen as unit of length. The angle also varies with the temperature, which must consequently be determined for each observation.

For the comparison of the rotatory power of different substances, use is made of the constant *specific rotation*, which may be defined as the angle of rotation, produced by 1 gram of active substance in 1 c.c. by a layer 1 dcm. in length. This is obtained by dividing the observed angle of rotation by the product of the length in decimetres l and the density d; or, if a weighed quantity of substance is taken, d will stand for the weight in 1 c.c. of solution.

$$[\alpha]^t_D = \frac{\alpha'_D}{l \times d}$$

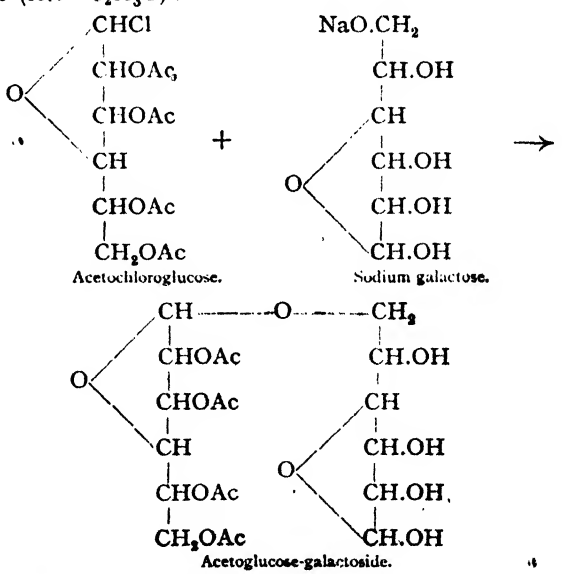
Example.—11·320 grams of cane-sugar were made up to 500 c.c. and introduced into a 2-decimetre polarimeter tube. After focussing the eye piece the zero point was determined. On introducing the sugar solution the difference between the first and second reading was + 3·07°.

Now $[\alpha]_D^{20}$ for cane-sugar $= +65{\cdot}9°$.

Therefore, sugar in 500 c.c. $\frac{3{\cdot}07 \times 500}{65{\cdot}5 \times 2} = 11{\cdot}335$ grams.

The percentage is $\frac{11{\cdot}335 \times 100}{11{\cdot}320} = 100{\cdot}1$

Synthesis of Disaccharoses.—Many attempts have been made to prepare the natural disaccharoses by combining molecules of the monosaccharoses; but so far with doubtful success. One method, examined by Fischer, was to act on glucose with hydrochloric acid. The product was not maltose, but isomaltose, an isomeric compound possessing many of its properties. Another method was to utilise the enzymes, maltase and emulsin, which as catalysts can act reversibly (see p. 103). The action of maltase on glucose gave isomaltose and some maltose, that of emulsin gave a second isomeric maltose (gentiobiose). A third synthetic method was to combine acetochloro-derivatives of the monosaccharoses with the sodium derivatives of these substances. Thus, acetochloro- or bromo-glucose has been combined with sodium galactose (Ac. $= C_2H_3O$):



The disaccharose obtained after removing the acetyl groups was not, however, natural lactose, but an isomeric compound. The same process has been adopted in other cases, but the products are not identical with the natural substances.

The Natural Glucosides.—This group of substances includes a variety of crystalline and, for the most part, colourless compounds of vegetable origin, which break up on boiling with mineral acids, or, by the action of specific enzymes, into a sugar and into a second organic constituent. The sugar is, as a rule, *d*-glucose, but glucosides containing both active forms of arabinose, *d*-xylose, *d*-ribose, *d*-galactose, *d*-mannose and *d*-fructose, as well as di- and tri-saccharoses are known (see p. 104).

Occasionally polyhydric alcohols may take the place of the sugar. The second constituent is usually an aromatic compound and includes a variety of phenols, aromatic alcohols, phenolic and hydroxy-acids, and substances such as ordinary mustard oil, purine compounds (p. 61), alkaloids, etc. The structure is probably similar to that of the simple alkyl glucosides, and, like these, they are divisible into α and β series as already explained (p. 43).

The functions of the glucosides in plants are at present somewhat obscure, but it is probable that they serve various purposes and that the enzyme which is commonly associated with the glucoside hydrolyses it at the required moment and so stimulates some important change in plant metabolism.

Among the substances which have in recent years been added to the list of glucosides is the tannin of galls. According to Fischer, the purified material from Chinese galls contains about 7–8 per cent. of *d*-glucose, to which it owes its activity. As gluco-gallic acid has been isolated from the galls he was led to conclude that tannin is probably a pentadigalloyl glucose.

$$[C_6H_2(OH)_3.CO.OC_6H_2(OH)_2.CO]_5.C_6H_7O_6$$

Pentadigalloyl glucose.

Compounds of this structure have been synthesised and have the properties of tannins.

CHAPTER IV

SOME NATURAL ORGANIC BASES

APART from the decomposition products of the proteins which are treated in Chapter V, the living organism (animal and vegetable) contains a number of basic substances, many of which possess considerable physiological interest.

When animal and vegetable tissues are treated with ether, in addition to fats, oils and cholesterol, small quantities of certain complex substances are extracted which are termed *lipins* or *phospholipins*; some yield galactose on hydrolysis and are known as *galactolipins*. Of these the best known are **lecithin** and **kephalin**, which break up on hydrolysis into glycerol, fatty acids (stearic, palmitic, oleic or linoleic), phosphoric acid, and the two amino-alcohols, **choline** and **amino-ethyl alcohol.**

They are therefore represented by the following formulæ, in which R stands for a fatty acid radical.

$$\begin{array}{l} CH_2(O.CO.R) \\ | \\ CH(O.CO.R) \\ | \\ CH_2.O.PO(OH).O.CH_2.CH_2.N(CH_3)_3.OH \end{array}$$

Probable formula of lecithin.

$$\begin{array}{l} CH_2(O.CO.R) \\ | \\ CH(O.CO.R) \\ | \\ CH_2.O.PO(OH).O.CH_2.CH_2.NH_2 \end{array}$$

Probable formula of kephalin.

Lecithin is present in nerve substance, brain, blood-corpuscles and egg-yolk. It is soluble in alcohol and ether, but insoluble in acetone, and is precipitated from an alcoholic solution by

cadmium chloride. It has a wax-like, crystalline appearance and forms salts with acids and a double salt with platinic chloride.

EXPT. 22.—**Preparation of Lecithin from Egg-yolk.**—Place in a flask an egg-yolk separated as far as possible from the white. Pour over it 20–30 c.c. of acetone, shake up, and let it stand with occasional shaking for an hour. Decant the yellow liquid and pour on a fresh quantity and decant, after standing, as before. After a third repetition most of the fat and cholesterol will be removed. Filter the protein precipitate which contains the lecithin at the pump, wash with a little acetone and press well down. Transfer the precipitate to a flask, pour on 50 c.c. of ether, cork the flask, and let the mixture stand overnight. Filter the ether solution and evaporate the ether which contains the lecithin to about 20 c.c. and add twice the volume of acetone, when a colourless, amorphous precipitate is thrown down. Filter, wash with a little acetone and re-dissolve in a small quantity of ether. Filter, if necessary, and re-precipitate with acetone as before. Filter and dry the precipitate in a vacuum desiccator. Lecithin is a colourless, wax-like substance. The yield is about 0·5 gram. The residue from the ether extract (about 5 grams) contains **vitellin** (p. 88), which is soluble in 8–10 per cent. salt solution and precipitated on dilution. The precipitate is also soluble in sodium carbonate solution and is re-precipitated on acidifying with acetic acid (see p. 88).

Choline is found in animal and vegetable cells either free or combined in the lipins, and is most readily obtained by hydrolysing lecithin with baryta solution and subsequently precipitating the base with alcoholic platinic chloride. Its structure has been determined by synthesis which has been effected by a variety of reactions. One method consists in acting upon ethylene chlorhydrin with trimethylamine, giving the chloride of the base (Renshaw),

$$(CH_3)_3N + ClCH_2.CH_2.OH = (CH_3)_3N\begin{matrix}Cl\\ \diagdown\\ CH_2.CH_2.OH\end{matrix}$$

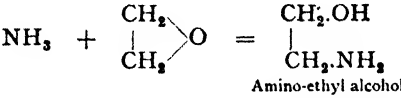
Choline chloride.

from which silver hydroxide liberates the base itself.

$$(CH_3)_3N\begin{matrix}Cl\\ \diagup\\ CH_2.CH_2.OH\end{matrix} + AgOH$$

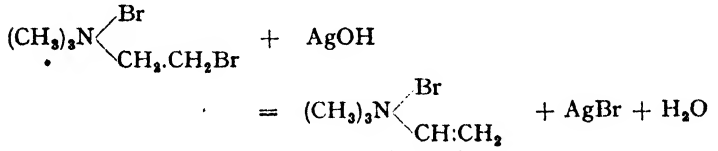
$$(CH_3)_3N\begin{matrix}OH\\ \diagup\\ CH_2.CH_2OH\end{matrix} + AgCl$$

Amino-ethyl alcohol.—The base is prepared from kephalin in a similar manner to that by which choline is obtained from lecithin. It has been synthesised by the action of ammonia on ethylene oxide.



Amino-ethyl alcohol.

Neurine is formed from choline by dehydration and is a product of animal putrefaction. Its structure is determined by its relation to choline and its synthesis from trimethylamine and ethylene bromide and subsequent treatment of the product with moist silver oxide.

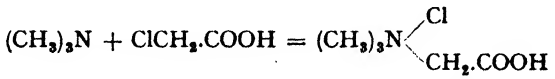


Neurine bromide.

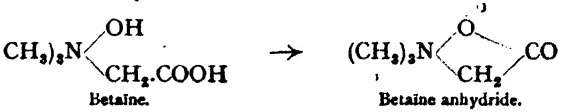
It is a powerfully toxic substance.

Betaïne is closely related chemically to choline. It is of widespread occurrence in plants and is frequently found in animal tissues. It is present in sugar-beet and accumulates in the molasses, which may contain as much as 6 per cent., and from which it is extracted by means of alcohol.

It has been prepared synthetically by the oxidation of choline and by the action of trimethylamine on monochloracetic acid, which yields the chloride of trimethyl glycine



Bases convert the latter into the hydroxide which on heating passes into the anhydride.



Betaïne. Betaïne anhydride.

Guanidine and Methylguanidine.—Although guanidine has occasionally been isolated from vegetable sources, its interest is chiefly determined by its presence as a constituent of creatine, certain xanthine bases (guanine) and protein cleavage products (arginine). It was originally prepared by the oxidation of guanine (p. 62), but is most conveniently obtained from ammonium thiocyanate.

EXPT. 23.—**Preparation of Guanidine.**—Heat 50 grams of ammonium thiocyanate to 180–190° in a 200 c.c. round flask in an oil- or metal-bath for twenty-four hours. Extract the yellowish mass with water, boil with a little animal charcoal, concentrate and filter the hot solution. Colourless crystals of guanidine thiocyanate separate; m.p. 117–118°. It may be purified by recrystallisation from water or alcohol. The yield is 20–25 grams. To convert it into the carbonate, it is dissolved in a very little water and the theoretical quantity of potassium carbonate added (10 parts require 5·8 parts of K_2CO_3) and evaporated to dryness on the water-bath. The residue is extracted with boiling alcohol (30 parts), when guanidine carbonate remains and may be recrystallised from water. (Volhard.)

Methylguanidine is physiologically of greater interest as it forms a normal constituent of muscle. It may be obtained by the oxidation of creatine or synthetically from cyanamide and methylamine (see p. 19).

$$NH_2.CN + NH_2.CH_3 = NH_2.C\begin{matrix}=NH\\ \diagdown NH.CH_3\end{matrix} \quad \text{or} \quad NH_2.C\begin{matrix}\diagup NH_2\\ =N.CH_3\end{matrix}.$$

Methyl guanidine.

Creatine and Creatinine.—Creatine is a constituent of all vertebrate muscle, and is found in the juice of flesh or meat extract to the extent of about 6 per cent. It yields methyl glycine or **sarcosine** and urea on hydrolysis with baryta:

$$\begin{matrix}CH_2.N\begin{matrix}\diagup CH_3\\ \diagdown C(:NH)NH_2\end{matrix}\\ |\\ COOH\end{matrix} + H_2O = \begin{matrix}CH_2.NH.CH_3\\ |\\ COOH\end{matrix} + CO\begin{matrix}\diagup NH_2\\ \diagdown NH_2\end{matrix}.$$

Creatine. Sarcosine. Urea.

On the other hand, sarcosine, on heating with cyanamide, in alcoholic solution yields creatine (Volhard).

$$\begin{array}{l} CH_2.NH.CH_3 \\ | \\ COOH \end{array} + CN.NH_2 = \begin{array}{l} CH_2.N\langle^{CH_3}_{C(:NH).NH_2} \\ | \\ COOH \end{array}.$$

On heating, or by treatment with dehydrating agents, creatine passes into creatinine.

$$\begin{array}{l} CH_2.N\langle^{CH_3}_{C(:NH)NH_2} \\ | \\ COOH \end{array} \rightarrow \begin{array}{l} CH_2.N(CH_3).C:NH \\ | \qquad\qquad | \\ CO\text{———}NH \end{array}$$

Creatine. Creatinine.

By the reverse process, under the action of alkalis creatinine is converted into creatine. Creatinine is absent from muscle, but is a normal constituent of the urine of mammals. Both substances occur in cereals and other crops.

EXPT. 24.—**Preparation of Creatine from Meat.**—Five hundred grams of meat, separated as far as possible from fat, are put through a sausage machine or finely chopped and digested with half a litre of water at 50–60°, and well stirred from time to time. It is filtered through cloth spread over a wooden frame (see Fig. 8), and is then digested with a further 250 c.c. of water in the same way filtered, and the cloth removed from the frame and squeezed into the filtrate. The filtrate is heated to boiling to coagulate the protein, and, on cooling, filtered. Basic acetate of lead (prepared by boiling a solution of lead acetate with excess of litharge (PbO) and filtering when cold) is carefully added, just sufficient to precipitate the soluble protein. The liquid is again filtered through a fluted filter (Vol. I, p. 33), and the lead removed with

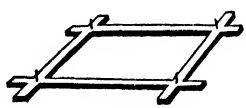


FIG. 8.

FIG. 9.—Creatine crystals (Krukenberg, after Kühne).

hydrogen sulphide, which is passed into the warm liquid. The filtrate from the lead sulphide is concentrated to a thin syrup on the water-bath, and then transferred to a vacuum desiccator, where it is left over sulphuric acid. In a short time, more quickly on the addition of a crystal of creatine, needle-shaped crystals begin to separate, and, when no further crystallisation is observed, the crystals, which have a brown colour, are brought on to a porcelain funnel and washed with a little spirit. They are recrystallised from a little hot water with the addition of animal charcoal. The yield is about 1 gram. The filtrate from the creatine contains a small quantity of hypoxanthine (p. 62) and sarcolactic acid (Vol. I, p. 171).

Two other basic products of importance are found among vital products, namely, **hordenine**, which is present in barley germs, and **adrenaline**, which occurs in the suprarenal gland.

Hordenine is most conveniently obtained from malt germs by extraction with alcohol, which is evaporated and the residue treated with water, the aqueous extract being then shaken with ether, which removes the base. It is a crystalline substance which melts at 118° and can be distilled under reduced pressure without decomposition. It has been prepared synthetically from phenyl ethyl alcohol by the following series of changes (Barger).

$C_6H_5.CH_2.CH_2.OH \rightarrow C_6H_5.CH_2.CH_2.Cl \rightarrow$
Phenyl ethyl alcohol. Phenyl ethyl chloride.

$C_6H_5.CH_2.CH_2.N(CH_3)_2 \rightarrow NO_2.C_6H_4.CH_2.CH_2.N(CH_3)_2 \rightarrow$
Phenyl ethyl dimethylamine. Nitrophenyl ethyl dimethylamine.

$NH_2.C_6H_4.CH_2.CH_2.N(CH_3)_2 \rightarrow HO.C_6H_4.CH_2.CH_2.N(CH_3)_2.$
Aminophenyl ethyl dimethylamine. Hordenine.

Hordenine is therefore a dimethyl *p*-hydroxyphenylethylamine. It has slight toxicity and when injected intravenously produces a feeble increase of blood pressure.

Adrenaline is separated from the suprarenal glands by water and is purified by precipitating foreign substances successively with neutral lead acetate and alcohol, in which the adrenaline

is soluble. From the concentrated solution it is finally precipitated by strong ammonia. Its principal physiological action when injected intravenously is to increase the blood-pressure (pressor action) by constricting the small arteries. It also accelerates the action of the heart. It is a colourless, crystalline substance, is lævo-rotatory, and melts at 211–212°. It has been synthesised from catechol by condensing it by the Friedel-Crafts' reaction with chloracetyl chloride. The chloracetyl derivative is then treated with methylamine and the product reduced either with the aluminium-mercury couple or by electrolysis.

$$(OH)_2C_6H_3.CO.CH_2Cl \rightarrow (OH)_2C_6H_3.CO.CH_2.NH.CH_3 \rightarrow (OH)_2.C_6H_3.CH(OH).CH_2.NH.CH_3$$

Adrenaline.

The product is *r*-adrenaline. It has been resolved by extracting the bitartrate with methyl alcohol, which dissolves the dextro-salt. The *d*-adrenaline is much less active than the laevo-compound. Many synthetic substitutes have been recommended but none have the activity of the natural product (see p. 142).

Glycocholic acid, $C_{26}H_{43}O_6N$, is present in ox bile as the sodium salt, and also in human bile, and is converted by hydrolysis with acids into cholic acid and glycine.

$$\underset{\text{Glycocholic acid.}}{C_{26}H_{43}O_6N} + H_2O = \underset{\text{Cholic acid.}}{C_{24}H_{40}O_5} + NH_2.CH_2.COOH$$

EXPT. 25.—**Preparation of Glycocholic acid from Ox-gall.**—Mix together in a basin the contents of an ox-gall with sufficient clean sand (about 200 grams) to produce on evaporation on the water-bath a hard, easily powdered mass. The dry powdered material is extracted with absolute alcohol on the water-bath, and the greenish-yellow solution filtered and washed, with hot alcohol, and the latter then removed as far as possible by distillation in the water-bath. Dissolve the sticky residue in a little water and add a small quantity of milk of lime to precipitate some of the colouring matter and filter. To the filtrate, which has now a pale greenish-yellow colour, add dilute sulphuric acid until a permanent turbidity is produced and set aside. Calcium sulphate crystallises and is separated by filtration. The process of adding sulphuric acid is repeated, when, after standing some time, a viscid liquid separates which in course of

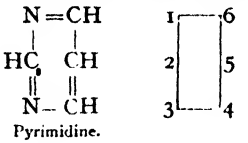
time (sometimes several days) becomes permeated with a mass of colourless crystals of glycocholic acid. It is filtered, washed with water, pressed down, spread on a porous plate to remove the sticky material as far as possible, and re-crystallised from hot water. It crystallises in clusters of needles. It is slightly soluble in cold water, more so in hot water, and very soluble in alcohol. It is insoluble in ether and acetone. It softens at 133° and melts at 152°, has a bitter taste and is dextrorotatory.

CHAPTER V

THE PYRIMIDINE AND PURINE GROUPS

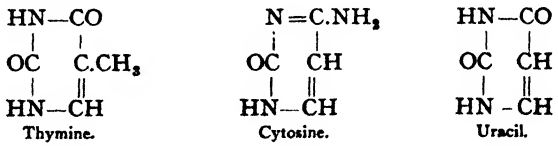
THE substances which form the subject of the present chapter are, like the preceding, normal constituents of the animal and vegetable organism, but belong to the class of heterocyclic compounds (Vol. I, p. 311).

Pyrimidine itself is a 6-atom ring of the following structure:



Pyrimidine.

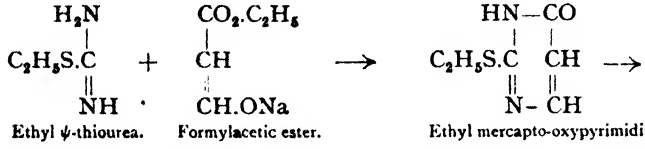
The positions of groups in the pyrimidine derivatives are numbered as in the annexed figure. It is not a natural product, but three of its derivatives, namely, 5-methyl 2 : 6-dioxypyrimidine or **thymine**, 2-oxy-6-aminopyrimidine or **cytosine**, and 2 : 6-dioxypyrimidine or **uracil**, are important constituents of the nucleic acid of cells (p. 89).



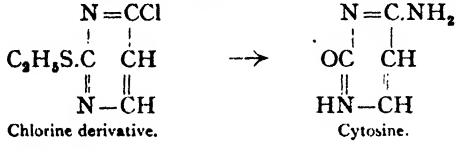
Thymine. Cytosine. Uracil.

All these substances have been obtained synthetically by more than one method. Wheeler and Johnson employ the following process. Ethyl ψ-thiourea and sodium formylacetic ester condense in aqueous solution with the formation of ethyl mercapto-oxy-

pyrimidine which, when treated with phosphorus pentachloride, yields a chlorine derivative. By the action of alcoholic ammonia on the latter the chlorine is replaced by the amino-group, and, on hydrolysis with hydrobromic acid, mercaptan is removed and cytosine is formed:



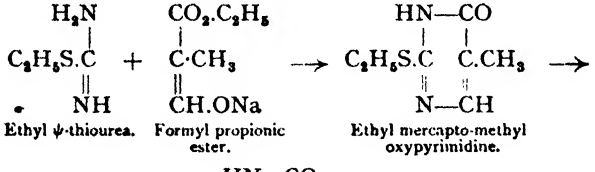
Ethyl ψ-thiourea. Formylacetic ester. Ethyl mercapto-oxypyrimidine.



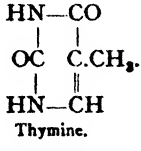
Chlorine derivative. Cytosine.

Cytosine crystallises in colourless plates containing 1 mol. H_2O, which it loses at 100°. It forms salts with acids, the principal being the picrate, which turns brown and melts at 270°. With nitrous acid it is converted into uracil.

In a similar manner to the above, ethyl ψ-thiourea and formyl-propionic ester yield a condensation product which, on boiling with hydrobromic acid, removes ethyl mercaptan and gives thymine.



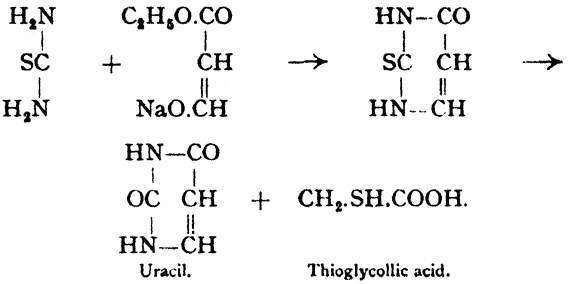
Ethyl ψ-thiourea. Formyl propionic ester. Ethyl mercapto-methyl oxypyrimidine.



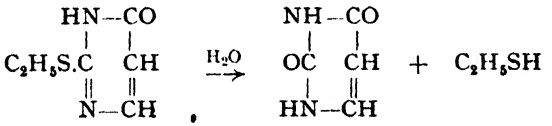
Thymine.

Thymine crystallises in rosettes or needles which dissolve readily in hot water, but not in cold. It does not form salts with acids, but combines with silver nitrate, forming a compound which is precipitated by ammonia. It melts at 321°.

Uracil is most conveniently synthesised by condensing thiourea with sodium formyl acetic ester and then exchanging the sulphur for oxygen by means of chloracetic acid, which forms uracil and thioglycollic acid :

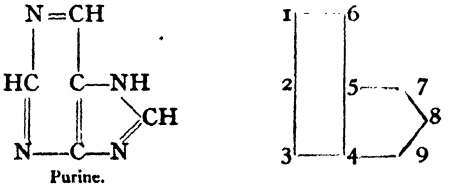


It may also be obtained from ethyl mercapto-oxypyrimidine (see above) by boiling with hydrobromic acid:



Uracil crystallises in clusters of needles which dissolve with difficulty in cold water. It behaves like thymine towards silver nitrate and ammonia.

Purine, like pyrimidine, is the parent substance of a number of natural products, but is not itself present in the living organism :

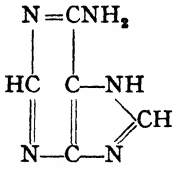


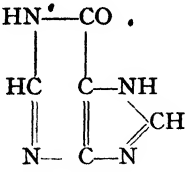
Purine.

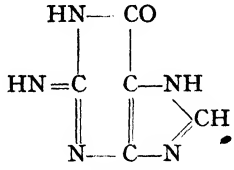
The positions of the groups in the numerous derivatives are indicated by the numbers in the adjoining figure.

6-Aminopurine or **adenine,** 6-oxypurine or **hypoxanthine,**

and 2-imino-6-oxypurine or **guanine** are among the products of hydrolysis of nucleic acids (p. 89):


Adenine.


Hypoxanthine.


Guanine.

Xanthine or 2 : 6-dioxypurine and many of its methyl derivatives are widely distributed among animal and vegetable substances. The structure of these substances will be presently discussed (p. 67). The following is a list of the natural xanthine bases and the sources from which they are derived.

NATURAL XANTHINE BASES.

Name.	Synonym.	Source.
Xanthine	—	Animal tissues
1-Methylxanthine . .	—	Urine
7-Methylxanthine . .	Heteroxanthine	Urine
1 : 3-Dimethylxanthine	Theophylline	Tea
1 : 7-Dimethylxanthine	Paraxanthine	Urine
3 : 7-Dimethylxanthine	Theobromine	Cocoa
1 : 3 : 7-Trimethylxanthine . . .	Caffeine	Tea, coffee, etc.

These substances are weak bases which form unstable salts with acids. They also combine with silver nitrate and other metallic salts, giving metallic derivatives.

Uric acid.—In addition to, and closely related to, the above are uric acid and its methyl derivatives (see Vol. I, p. 228), the structure and synthesis of which will now be discussed. It has already been pointed out that on oxidation uric acid breaks up into alloxan and urea.

$$\underset{\text{Uric acid.}}{C_5H_4O_3N_4} + O + H_2O = \underset{\text{Alloxan.}}{C_4H_2O_4N_2} + \underset{\text{Urea.}}{CO(NH_2)_2}$$

Intermediate products in the oxidation of uric acid are alloxantin and allantoin, the latter being found in the allantoic fluid of the calf and sometimes in the urine after administration of uric acid. Uric acid crystallises in a variety of forms as shown in Fig. 10.

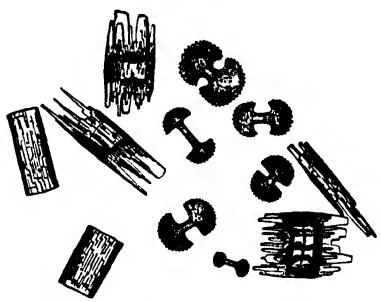


FIG. 10.—Crystals of uric acid (after Funke).

The quantity is estimated by oxidation with potassium permanganate.

EXPT. 26.—**Estimation of Uric acid.**—Weigh out accurately about 0·5 gram of uric acid, wash it into a litre flask and add about 0·5 gram of potassium hydroxide, when a clear solution should be obtained. Make up to 1 litre. Prepare a $N/20$ solution of potassium permanganate (1·58 grams $KMnO_4$ in 1 litre; 1 c.c. = 0·00375 gram of uric acid) and fill up the burette. Measure out 100 c.c. of the uric acid solution, add 20 c.c. of conc. sulphuric acid, shake up and immediately titrate with the permanganate solution, stirring or shaking gently during the process, until a drop of permanganate gives a pink colour which is diffused through the solution. The titration is then complete, though the colour may shortly disappear.

Example.—100 c.c. = 12·5 c.c. of permanganate solution

$$\frac{12{\cdot}5 \times 0{\cdot}00375 \times 100}{0{\cdot}05} = 93{\cdot}7 \text{ per cent.}$$

EXPT. 27.—**Preparation of Alloxantin from Uric acid.**—Mix 18 c.c. of conc. hydrochloric acid with an equal volume of water and add it to 10 grams of uric acid in a beaker. Heat the mixture to 35° and add 2·5 grams of finely powdered potassium chlorate in small quantities at a time with constant shaking. When about 2 grams of the chlorate have been added, the uric acid will have nearly dissolved, and the liquid become faint yellow. When all the chlorate has been introduced the mixture is diluted with double its volume of water, left for an hour and filtered. The filtrate is saturated with hydrogen sulphide, and after being left for twelve hours, deposits crystalline crusts often of a reddish tinge, consisting of alloxantin mixed with sulphur. It is filtered and washed with cold water, and the alloxantin dissolved in a

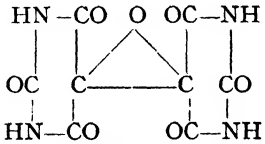
small quantity of hot water and filtered from sulphur. On cooling the filtrate, colourless crystals separate. The yield is 7–8 grams.

The formation of alloxantin is due to the formation of alloxan by oxidation, which unites with a second and partially reduced molecule:

$$\underset{\text{Uric acid.}}{C_5H_4O_3N_4} + O + H_2O = \underset{\text{Alloxan.}}{C_4H_2O_4N_2} + CO(NH_2)_2$$

$$2C_4H_2O_4N_2 + H_2S = \underset{\text{Alloxantin.}}{C_8H_4O_7N_4} + S + H_2O$$

The structural formula of the latter is probably the following:



Alloxantin.

Add to the solution of alloxantin a little baryta water; a violet coloration is produced; add ammonia-silver nitrate solution and warm; metallic silver is deposited. Boil the solution with mercuric oxide, a violet solution of murexide is formed.

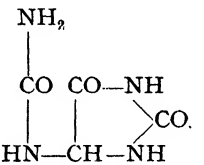
EXPT. 28.—**Preparation of Allantoin from Uric acid.**—To 5 grams of uric acid contained in a flask ($\frac{1}{2}$ litre) add enough water to form a thin paste and about 1 gram of sodium hydroxide dissolved in a little water, place the flask in ice-water and, whilst cooling, dissolve 3·2 grams of potassium permanganate in 150 c.c. of water. Add the cooled permanganate solution a few c.c. at a time to the uric acid, shake well and keep the temperature below 10°. The permanganate is quickly reduced and manganese dioxide is precipitated. When the whole of the permanganate has been added and the

FIG. 11.—Crystals of allantoin prepared by the oxidation of uric acid (after Kühne).

pink colour gone, pass in a current of sulphur dioxide, keeping the flask in the ice-water, and shake well until the precipitate of manganese dioxide is just dissolved. Filter from any unchanged uric acid, if necessary, and concentrate the solution to about half its bulk on the water-bath. Set aside and let it stand for about two days, when colourless, crystalline crusts of allantoin are deposited. The yield is theoretical. The substance may be recrystallised from hot water, and has the appearance shown in Fig. 11 (Claus).

$$\underset{\text{Uric acid.}}{C_5H_4O_3N_4} + H_2O + O = \underset{\text{Allantoin.}}{C_4H_6O_3N_4} + CO_2.$$

It crystallises in shining prisms with dome-shaped ends, and has the following structure:



Allantoin.

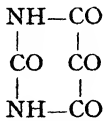
EXPT. 29.—**Preparation of Alloxan from Alloxantin.**—Add 5 grams of finely powdered alloxantin to a mixture of 3·5 c.c. of conc. nitric acid (sp. gr. 1·4) and 7 c.c. of fuming nitric acid (sp. gr. 1·5), and leave it for about two days. Slight evolution of nitrous fumes occurs, and the alloxantin, which first remains at the bottom of the vessel, slowly changes into the more bulky crystals of alloxan, which gradually fill the liquid. The reaction is complete when a sample of the product dissolves readily in cold water. The crystalline mass is filtered and spread upon a porous plate, thoroughly dried in the air, and freed from traces of nitric acid by heating in a basin on the water-bath, until the smell of the acid disappears. It may be obtained in large crystals by dissolving the product in the smallest quantity of hot water and allowing the solution to evaporate slowly in a desiccator over sulphuric acid. The crystals are liable to effloresce in the air.

$$C_8H_4O_7N_4 + O = 2C_4H_2O_4N_2.$$

The crystals contain 4 molecules of water of crystallisation. On evaporating a solution of alloxan to dryness on the water-

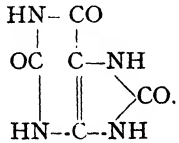
bath, a reddish residue is left which turns purple on the addition of ammonia (murexide).

The following formula has been assigned to alloxan:



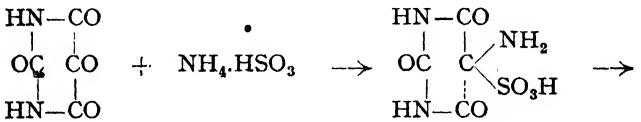
Alloxan.

By combining the formula of alloxan with that of urea (with loss of two atoms of hydrogen and oxygen) a structure for uric acid is obtained which explains the above decomposition as well as the existence of the various mono-, di-, tri- and tetra-methyl uric acids.

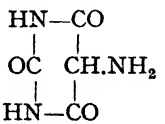


Uric acid.

Synthesis of Uric acid.—The substance is therefore 2 : 6 : 8-trioxypurine. Its synthesis has been effected in various ways, of which the simplest is that devised by Fischer. Alloxan combines with ammonium bisulphite to form **thionuric acid**, which, on hydrolysis, loses sulphuric acid and yields **uramil.**

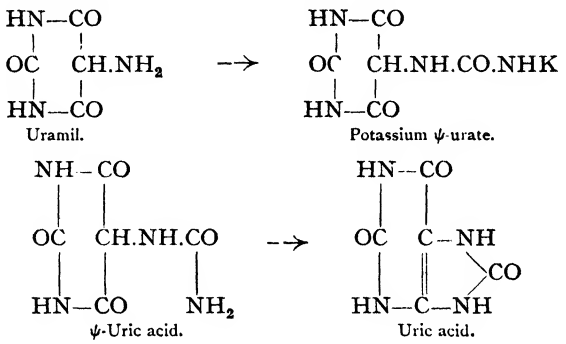


Alloxan. Thionuric acid.



Uramil.

The latter combines with potassium cyanate, giving the potassium salt of ψ-uric acid, which on heating with hydrochloric acid loses the elements of water and yields uric acid.



In a similar way, dimethyl alloxan has been converted into 1 : 3-dimethyl uric acid and dimethyl alloxan and methylamine bisulphite into 1 : 3 : 7-trimethyluric acid.

Structure of Xanthine bases.—The relation of the xanthine bases to uric acid is clearly indicated by the fact that xanthine and uric acid have closely related formulæ :

Uric acid $C_5H_4O_3N_4$
Xanthine $C_5H_4O_2N_4$,

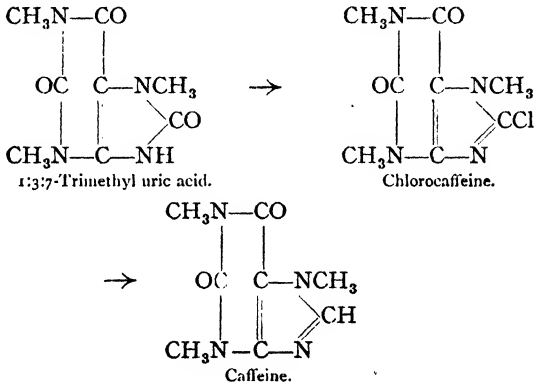
and that xanthine gives on oxidation the same products as uric acid, namely, alloxan and urea. Theobromine in the same way yields methyl alloxan and methyl urea, and is therefore a dimethyl xanthine, whilst caffeine breaks up into dimethyl alloxan and methyl urea, and is a trimethyl xanthine. Moreover, xanthine can be converted into theobromine by methylating with methyl iodide and sodium hydroxide solution, and theobromine into caffeine by the further action of the same reagents. The relationship of these three substances is therefore

$C_5H_4O_2N_4$	$C_5H_2(CH_3)_2O_2N_4$	$C_5H(CH_3)_3O_2N_4$
Xanthine.	Theobromine.	Caffeine.

It would appear from these relationships that uric acid and its methyl derivatives could be transformed by reduction, *i.e.*, by the loss of an atom of oxygen, into the corresponding xanthine bases. No process of direct reduction has yet been effected. The method devised by Fischer is to convert the uric acids into

the halogen compounds by means of phosphorus chloride and to reduce the product with hydriodic acid.

Caffeine, the most important of these bases, is obtained from 1 : 3 : 7-trimethyl uric acid (prepared by the methylation of uric acid) as follows : the trimethyl uric acid is heated with a mixture of penta- and oxy-chloride of phosphorus, giving chlorocaffeine, which is then reduced to caffeine with hydriodic acid.



All the other natural purine bases, including purine itself, have been obtained synthetically, but many of the processes are too involved and complex to be described here.

The Formation of Uric acid in the Body.—The amount of uric acid excreted by different animals varies enormously. It forms the greater part of the nitrogenous constituents of the excreta of birds and reptiles, whilst in most mammals, including man, the proportion is only about 2 per cent. The origin of the uric acid in the two cases is very different. In the case of birds it would appear that the liver converts ammonia and urea into uric acid, whereas in mammals the contrary is the case and uric acid is transformed into urea. This function of destroying uric acid is generally ascribed to the so-called **uricolytic enzyme** of the liver. But, as the destruction is never complete, a small portion is always present in the urine. The uric acid found in the urine of mammals is partly derived from the purine constituents of nucleo-proteins (p. 89) (namely, xanthine, guanine, hypoxanthine and adenine)

by the agency of certain oxidising enzymes (oxidases, see p. 103), since food rich in nucleo-proteins increases the uric acid secretion. But seeing that uric acid is secreted during starvation, a portion must be derived from the metabolism of the tissues, that is, from the nucleo-proteins of the disintegrated cells, and is therefore independent of the food supply.

A third source of uric acid is probably the product of normal cell metabolism of the tissues; for hypoxanthine is always present with creatine and lactic acid in the juice of muscle, and is converted by oxidation into uric acid.

The synthesis of the purine bases within the organism is still involved in obscurity. That the living cell can effect this synthesis is proved by the presence of nucleo-proteins in young animals from food containing no purine derivatives.

CHAPTER VI

THE PROTEINS

THE general properties of the proteins and their reactions have been described in Vol. I, p. 235. In the present chapter it is proposed to consider in greater detail the nature of their hydrolytic products and their possible structure.

Products of Protein hydrolysis.—Hydrolysis may be effected by boiling with mineral acids (hydrochloric or sulphuric acid), or alkalis, or by the action of certain enzymes such as pepsin, trypsin, and erepsin (p. 102). The products are a variety of amino-acids, which are classified as follows:

Monobasic Monoamino-acids.

Glycine = aminoacetic acid, $(NH_2)CH_2.COOH$.

Alanine = α-aminopropionic acid, $CH_3.CH(NH_2).COOH$.

Valine = α-aminoisovaleric acid, $\begin{matrix}CH_3\\CH_3\end{matrix}\!\!>\!CH.CH(NH_2).COOH$.

Leucine = α-aminoisobutyl acetic acid,

$$\begin{matrix}CH_3\\CH_3\end{matrix}\!\!>\!CH.CH_2.CH(NH_2).COOH.$$

Isoleucine = secondary butyl α-aminoacetic acid, $\begin{matrix}CH_3\\C_2H_5\end{matrix}\!\!>\!CH.CH(NH_2).COOH$.

Caprine = α-aminocaproic acid,

$$CH_3.CH_2.CH_2.CH_2.CH(NH_2).COOH.$$

Dibasic Monoamino-acids.

Aspartic acid = α-aminosuccinic acid, $\begin{matrix}CH(NH_2).COOH\\ |\\ CH_2.COOH\end{matrix}$.

Glutamic acid = α-aminoglutaric acid, $\begin{matrix}CH(NH_2).COOH\\ |\\ CH_2.CH_2.COOH\end{matrix}$.

Hydroxy- and -Thioamino-acids.

Serine = α-amino-β-hydroxypropionic acid,
$CH_2(OH).CH(NH_2).COOH$.

α-Amino-β-hydroxyglutamic acid,
$COOH.CH_2.CH(OH).CH(NH_2).COOH$.

Trihydroxydiaminododecanic acid, $C_{11}H_{16}(OH)_3(NH_2)_2.COOH$.

Cysteine = α-amino β-thiolactic acid, $CH_2(SH).CH(NH_2).COOH$

Cystine = dithioaminopropionic acid, $\begin{matrix}S.CH_2.CH(NH_2).COOH\\ |\\ S.CH_2.CH(NH_2).COOH\end{matrix}$.

Diamino-acids.

Ornithine = $\alpha\delta$-diaminovaleric acid,
$(NH_2)CH_2.CH_2.CH_2.CH(NH_2).COOH$.

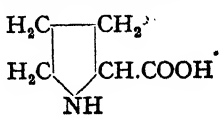
Lysine = $\alpha\epsilon$-diaminocaproic acid,
$(NH_2)CH_2.CH_2.CH_2.CH_2.CH(NH_2).COOH$.

Arginine = α-amino-δ-guanidovaleric acid,

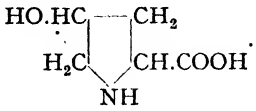
$$(NH_2)\overset{\overset{NH}{\|}}{C}—NH.CH_2.CH_2.CH_2.CH(NH_2).COOH$$

Heterocyclic Amino-acids.

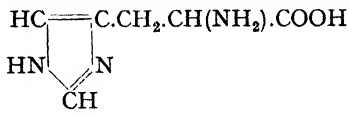
Proline = α-pyrrolidine carboxylic acid,



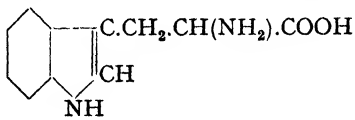
Oxyproline = hydroxypyrrolidine carboxylic acid,



Histidine = α-amino-β-iminazole propionic acid,

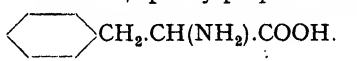


Tryptophane = indole-α-aminopropionic acid,

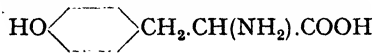


Aromatic Amino-acids.

Phenylalanine = α-amino-β-phenylpropionic acid,



Tyrosine = α-amino-p-hydroxyphenylpropionic acid,



It will be seen from the above table that there are about twenty different amino-acids, the majority of which are commonly ong the products of hydrolysis of typical proteins, s we shall presently see, their relative quantities vary

ucts of hydrolysis have been synthesised and their sequently fully established.

n of certain of these products from their natural be described, and then a few of the various will be illustrated.

e **ester hydrochloride from Gelatine.**— mercial gelatine or size with 300 c.c. of and shake until the gelatine is nearly

dissolved; then add a few fragments of porous pot and boil over wire-gauze with reflux condenser for four hours. The dark coloured product is now evaporated on the water-bath under diminished pressure in the apparatus shown in Fig. 12.

It consists of two distilling flasks (1 litre) fitted together by rubber corks, the one acting as distilling flask and the other as receiver. The receiver, which is cooled by a stream of water, is attached to a water-jet aspirator. A long capillary, which nearly touches the bottom of the flask, is inserted through the cork of the distilling vessel. It serves to agitate the liquid by introducing a stream of fine air-bubbles which keep it in constant motion. When the water is removed as far as possible, the

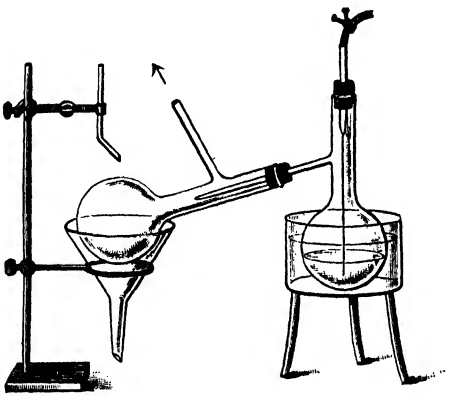


FIG. 12.

residue, which forms on cooling a thick, viscid mass, is mixed with 500 c.c. of anhydrous alcohol. It is heated on the water-bath with reflux condenser for a short time, with the addition of a little charcoal to remove the colour, and filtered. The alcoholic solution is cooled in ice and saturated with dry hydrogen chloride. The liquid is then boiled for half an hour on the water-bath, cooled, and, after dropping in a crystal of the substance, left overnight. Glycine ester hydrochloride crystallises in colourless needles, and is filtered and washed with a little alcohol. The yield is 10–15 grams; m.p. 144°.

Uramidoacetic ester.—Dissolve 1 gram of the glycine ester hydrochloride in 1 c.c. of water and 0·7 gram of potassium cyanate in 1 c.c. of water, mix the two solutions and concentrate on the water-bath till crystals begin to appear. On cooling,

uramidoacetic ester separates in octahedra or prisms. It may be re-crystallised from alcohol; m.p. 135°.

$$\begin{array}{l}CH_2.NH_2.HCl\\ |\\ COO.C_2H_5\end{array} + KOCN = \begin{array}{l}CH_2.NH.CO.NH_2\\ |\\ COO.C_2H_5\end{array} + KCl.$$

Glycine ester hydrochloride. — Uramidoacetic ester.

Hydantoin.—On heating on the water-bath with 25 per cent. hydrochloric acid for a quarter of an hour uramidoacetic ester is converted into hydantoin, which crystallises on cooling; m p. 217–219°. (Harries and Weiss.)

$$\begin{array}{l}CH_2.NH.CO.NH_2\\ |\\ COO.C_2H_5\end{array} = \begin{array}{l}CH_2.NH.CO\\ |\quad\quad\quad|\\ CO\text{———}NH\end{array} + C_2H_5.OH.$$

Hydantoin.

If the heating is continued, hydantoin is hydrolysed and gives glycine hydrochloride.

Other hydantoins may be obtained in the same way from the amino-acid ester hydrochloride or the amino-acid (p. 75).

EXPT. 31.—**Preparation of Hippuric acid from Urine.**—To 20–30 c.c. of the urine of a herbivorous animal add milk of lime until on boiling it remains alkaline. Filter from the white, flocculent precipitate and then concentrate the solution to about one-third of its original volume. On the addition of a little conc. hydrochloric acid to the cooled liquid, colourless needles of hippuric acid separate. After standing for a short time the crystals are filtered and may be purified by recrystallisation from a little hot water. They retain colouring matter, which is difficult to remove. The yield from 30 c.c. of goats' urine is about half a gram; m.p. 187–188°.

FIG. 13.—Hippuric acid crystals (after Funke).

EXPT. 32.—**Synthesis of Hippuric acid from Glycine.**—Glycine, obtained as described in Vol. I, p. 232, may be used. Dissolve 0·75 gram of glycine in 5 c.c. of water, add 1·25 grams of benzoyl chloride. Keep alkaline by occasionally adding a little sodium hydroxide solution, warm gently, and shake well until the smell of benzoyl chloride has disappeared. Add conc. hydrochloric acid until acid, and let stand a few hours. Filter and recrystallise from hot water. The yield is 1 gram; m.p. 187–188°.

EXPT. 33.—**Leucine and Tyrosine from Horn or Casein.**—Heat in a round flask (1½ litres) 100 grams of casein or hoof or horn shavings, washed free from dirt, with 136 c.c. of conc. sulphuric acid in 750 c.c. of water on the water-bath until the greater part is dissolved, and then boil over wire-gauze with reflux condenser for twenty hours until the solution no longer gives the biuret reaction (Vol. I, p. 235). After boiling, the dark coloured liquid is poured into a large basin and neutralised whilst hot with slaked slime. The hot liquid is filtered and the residual calcium sulphate replaced in the basin and extracted twice with 300 c.c. of hot water. The united filtrates are concentrated and made up to a litre. The total quantity of oxalic acid (about 20 grams) required to precipitate the dissolved calcium salts is determined by a preliminary estimation with 50 c.c. of the solution. The liquid is boiled before adding the acid and filtered hot from the precipitated calcium oxalate. The precipitate is extracted twice with 250 c.c. of water and concentrated to about 250 c.c. until crystals appear on the surface. On cooling, a brown, crystalline crust of impure tyrosine separates. It is filtered, dissolved in the least quantity of boiling water, boiled with a little animal charcoal and filtered. On cooling, long, white, silky needles of tyrosine are deposited. Yield about two grams.

Reactions.—Warm a small quantity of the substance with a drop of strong nitric acid and add ammonia. A yellow solution is produced with the acid and changes to deep orange with ammonia (xanthoproteic reaction). Warm with a solution of mercury in strong nitric acid (Millon's reagent). The liquid turns red and a red precipitate is formed.

Tyrosine Hydantoin.—Suspend 1 gram of tyrosine in 5 c.c. of boiling water and add about 0·5 gram of potassium cyanate until a clear solution is obtained. Then boil for a quarter of an hour with 10 c.c. of hydrochloric acid (1 conc. HCl : $2H_2O$), when a colourless, crystalline mass separates consisting of the hydantoin compound (Dakin).

$$HO.C_6H_4.CH_2.CH(NH_2).COOH + HOCN \rightarrow HO.C_6H_4.CH_2.CH(NH.CO.NH)CO$$

Tyrosine hydantoin.

The filtrate from the crude tyrosine contains leucine and is further concentrated on the water-bath to a small bulk, when, on cooling, a quantity (about 20 grams) of crude leucine, in the form of a brown, crystalline crust, separates and is collected on a filter and dried on a porous plate. It is purified by conversion into the ester hydrochloride as follows. The dry material is dissolved in 120 c.c. of absolute alcohol and saturated with hydrogen chloride. The alcohol is removed by distilling under reduced pressure, at a temperature not exceeding 40°, in the apparatus, shown in Fig. 12, p. 73. The same quantity of alcohol is added, saturated with hydrogen chloride, and removed as before. The residue, which consists of the ester hydrochloride of leucine and small quantities of other amino-acids, is converted into the free ester in the following way. It is dissolved in about one-quarter of its volume of water to which an equal volume of purified ether is added. The liquid is well cooled in a freezing mixture and a cooled 33 per cent. solution of sodium hydroxide is slowly added until the liquid is just alkaline, followed by an equal volume of a saturated solution of potassium carbonate. The mass is now well shaken and the ether decanted. In this way, the ester, which is rapidly hydrolysed by alkali at the ordinary temperature, is liberated from the hydrochloride without decomposition and dissolves in the ether. The residue is kept in the freezing mixture, a fresh quantity of ether, more sodium hydroxide solution, and sufficient solid potassium carbonate to

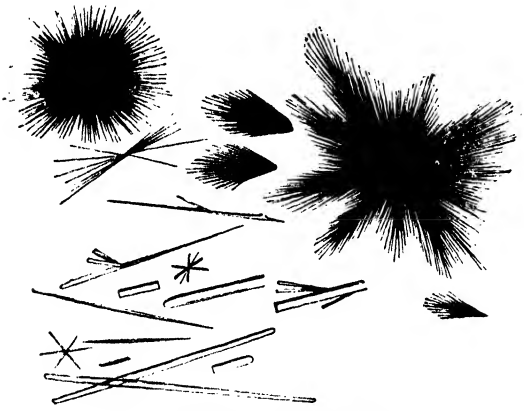


FIG. 14.—Tyrosine crystals (Krukenberg)

form a pasty mass are added in succession, shaken up thoroughly, and the ether decanted. The residue is extracted two or three times with fresh ether and the united extract, freed as far as possible from water, is shaken up for a minute with solid potassium carbonate and then dehydrated overnight with anhydrous sodium sulphate. The ether is removed on the water-bath and the residue distilled at a pressure not exceeding 15 mm. The colourless liquid, which distils at 80–100°, has an ammoniacal smell and is nearly pure leucine ester. The yield is 10–15 grams. The ester is readily hydrolysed by boiling it with five times its weight of water under a reflux condenser until the alkaline reaction disappears. The liquid is concentrated on the water-bath until crystals separate on the surface and then cooled. The leucine may be recrystallised from dilute alcohol, or dissolved in the smallest quantity of hot water, and alcohol added until a turbidity appears. It forms glistening plates which melt and sublime at 170°.

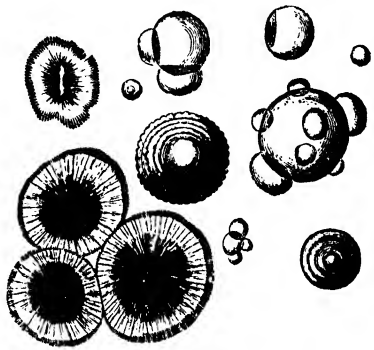


FIG. 15.—Leucine crystals (Krukenberg).

EXPT. 34.—**Preparation of Tryptophane from Casein.**—Rub up 200 grams of commercial casein with water and make up to 2 litres. Add 16 grams of anhydrous sodium carbonate, about 40 c.c. of an active trypsin solution (liquor pancreaticus, Benger), and 10 c.c. of chloroform, and digest at 35° in an incubator for seven days. At the end of the first or second day add a further 40 c.c. of trypsin solution. After incubation, heat to 80°, cool and filter through a large, fluted filter. To the filtrate add 50 c.c. of conc. sulphuric acid for each litre of solution and allow it to stand to precipitate calcium sulphate. Filter if necessary, and add mercuric sulphate solution, which is prepared as follows: 25 c.c. of conc. sulphuric acid are poured into 475 c.c. of water, and 50 grams of mercuric sulphate are ground in a mortar with successive portions of the dilute acid and poured into the main bulk of acid, in which the greater part dissolves. It is then allowed to settle and filtered. Add to the casein solution the

mercuric sulphate solution whilst briskly stirring until there is no further precipitate on standing (about 100 c.c.) and leave it for twelve hours. A lemon-yellow precipitate separates and is filtered at the pump and washed with 5 per cent. sulphuric acid until the filtrate no longer gives with Millon's reagent in the cold a pink, but only a yellowish coloration (absence of tyrosine). Suspend the precipitate in about 100 c.c. of water, heat the liquid to 50°, and pass in hydrogen sulphide. As the mercury is very slowly thrown down as sulphide, the process of heating and passing in hydrogen sulphide must be repeated at least three times. The mercuric sulphide is removed by filtration, and air is drawn through the filtrate until the smell of hydrogen sulphide disappears. The liquid is exactly neutralised with baryta solution, filtered, and concentrated in vacuo to a small bulk (about 30 c.c.). This solution is extracted in an apparatus, which is shown in the accompanying figure, 16, resembling a Soxhlet extractor (p. 23).

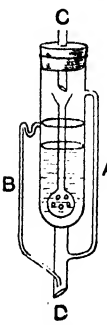


FIG. 16.

About 50 c.c. of butyl alcohol are placed in the flask attached to *D* and boiled. The vapour condenses at *C* and drips into the inner tube of the extractor, containing the solution of tryptophane, and emerges through the liquid, returning by the overflow *B* to the flask.

The tryptophane thus becomes concentrated in the butyl alcohol. On evaporating the solvent in vacuo, the tryptophane crystallises. The yield is 1–2 grams.

EXPT. 35.—**Preparation of Glutamic acid from Gluten.**—Knead in a large basin under a slow running stream of water 400–500 grams of flour. The starch is gradually removed and a sticky, yellowish mass of gluten remains. The process of kneading is continued as long as the water appears milky and the gluten contains lumps. Dry the gluten as far as possible on the water-bath, after squeezing out most of the water.

Boil 50 grams of the dry gluten in a round flask (1 litre) provided with an air condenser with 150 c.c. of conc. hydrochloric acid for four hours. Filter from black humus and wash with hot water. Evaporate the filtrate on the water-bath under diminished pressure as described on p. 73. A brown, sticky residue remains which on cooling deposits small crystals of glutamic acid hydrochloride. Warm gently with absolute alcohol on the water-bath with reflux condenser, and filter and wash with alcohol. Colourless

crystals of glutamic acid hydrochloride remain on the filter. The yield is about 2 grams.

EXPT. 36.—**Preparation of Cystine from Wool.**—Introduce into a 1½-litre round flask fitted with an air condenser 50 grams of wool free from grease and 100 c.c. of conc. hydrochloric acid, and boil gently for about three hours until the liquid no longer gives the biuret reaction (see Vol. I, p. 235). To the hot acid solution add powdered crystallised sodium acetate until Congo red paper is no longer coloured blue (about 120 grams), and let the mixture stand for several hours. A heavy, brown precipitate collects at the bottom of the vessel. Filter at the pump, wash with a little cold water and dissolve the dark brown residue in 40 c.c. of 5 per cent. hydrochloric acid, and boil with a little animal charcoal (previously digested with hydrochloric acid to remove calcium phosphate) until decolorised. To the hot filtrate sodium acetate is slowly added, when the cystine is precipitated as a colourless, or faintly coloured, crystalline powder. If coloured, a second treatment with hydrochloric acid and charcoal will remove the colour. The yield is 2·5 grams. A further quantity may be obtained from the mother liquor on evaporation. It is dissolved in dilute hydrochloric acid and precipitated by sodium acetate. (Folin.)

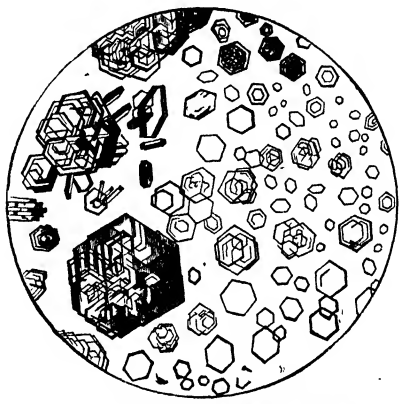


FIG. 17.—Cystine crystals (after Funke).

The following general methods have been used in the synthesis of amino-acids.

1. The action of ammonia on a halogen acid (Perkin and Duppa). This has already been illustrated in Vol. I, p. 232, in the case of glycine.

$$2NH_3 + ClCH_2.COOH = NH_2.CH_2.COOH + NH_4Cl.$$

2. The bromination of an alkyl malonic acid and removal of carbon dioxide on heating. This yields the monobasic α-bromo-acid, which, with ammonia, forms the amino-acid (Fischer).

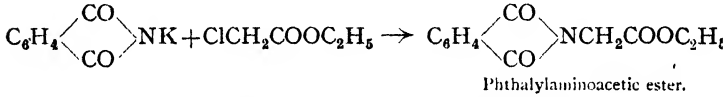
The process is illustrated in the preparation of phenylalanine (p. 81).

$$C_6H_5CH_2Cl + NaCH(COOC_2H_5) \rightarrow C_6H_5CH_2CH(COOC_2H_5)_2$$
Benzylmalonic ester.

$$\rightarrow C_6H_5CH_2CBr(COOH)_2 \rightarrow C_6H_5CH_2CHBrCOOH$$
Benzyl bromomalonic acid. Phenyl bromopropionic acid.

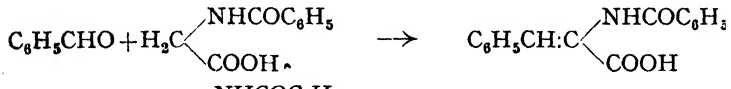
$$\rightarrow C_6H_5CH_2CH(NH_2)COOH$$
Phenylalanine.

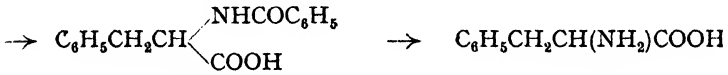
3. The interaction of potassium phthalimide and a halogen aliphatic ester. On hydrolysis, the amino-acid and phthalic acid are formed (Gabriel). Potassium phthalimide and chloracetic ester yield the phthalylaminoacetic ester, which on hydrolysis gives phthalic acid and glycine.


Phthalylaminoacetic ester.

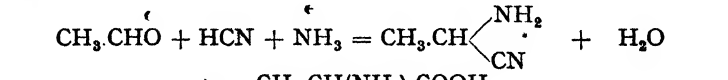
$$\rightarrow C_6H_4(COOH)_2 + NH_2CH_2COOH$$
Phthalic acid. Glycine.

4. The condensation of aldehydes with hippuric acid in presence of acetic anhydride and reduction and subsequent hydrolysis of the resulting product (Erlenmeyer). Benzaldehyde and hippuric acid may be converted into phenylalanine by this method by the following series of reactions:


Benzoylphenylalanine.


Phenylalanine.

5. A further method depends on the conversion of the aldehyde cyanhydrins into the aldehyde ammonia cyanhydrin and the hydrolysis of the latter, which yields the amino-acid (Strecker). Acetaldehyde may be converted in this way into alanine.


Alanine.

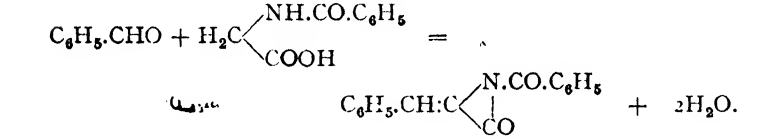
These methods will now be illustrated in detail by the following series of experiments.

EXPT. 37.—**Synthesis of Phenylalanine from Malonic ester.**—Dissolve 4·6 grams of metallic sodium in 20 c.c. of pure alcohol, and, whilst warm, add 32 grams of malonic ester. Cool and add 25 grams of benzyl chloride and heat the mixture for twelve hours in a soda-water bottle with the cork well wired in and placed in a water-bath. Test the product, which should be neutral to litmus, by withdrawing a small sample, evaporating the alcohol, and dissolving the residue in water. Distil off as much alcohol as possible from the water-bath, in which the distilling flask should be immersed, add a little water and extract with ether. Dehydrate the ether solution with fused calcium chloride, distil off the ether and distil the ester in vacuo. At about 25 mm. it boils at 190–200°. The yield is about 22 grams. To saponify the ester, heat on the water-bath 20 grams with a solution of potassium hydroxide (made by dissolving 12 grams of the hydroxide in 20 c.c. of water). Heat on the water-bath for several hours when saponification should be complete. This is determined by withdrawing a sample, and adding water, when a clear solution should be obtained. To remove any unchanged ester add water and shake out once or twice with ether. Add a little conc. hydrochloric acid until acid, and extract the benzylmalonic acid several times with small quantities of ether. Dehydrate the ether over fused sodium sulphate, distil off most of the ether, pour out the residue into a basin and remove the remainder of the ether on the water-bath. On standing, the substance crystallises; m.p. 116°. The yield is 12 grams.

Ten grams of benzylmalonic acid are dissolved in 50 grams of dry ether and 10 grams (3·3 c.c.) of bromine gradually added. Exposed to daylight, the bromine disappears rapidly at first and hydrogen bromide is evolved. Towards the end of the reaction the liquid retains the brown colour of the bromine. After standing half an hour, the ether solution, with the addition of a small quantity of water, is treated gradually with sulphur dioxide until, on shaking, the colour disappears and the ether layer is then removed, washed with a little water, and then carefully evaporated, and the solid residue crystallised from 50 c.c. of hot benzene. The yield is about 12 grams; m.p. 137°. The moist benzylbromomalonic acid is now heated in the oil-bath to 125–130°, whereby carbon dioxide and a little hydrogen bromide are evolved. After three-quarters of an hour the reaction is complete, and the

residual yellow oil is washed with water, dissolved in ether, and after dehydrating over anhydrous sodium sulphate, the ether is removed. The residual, mobile and nearly colourless oil is dissolved in five times its weight of 25 per cent. aqueous ammonia, and left for three to four days at the ordinary temperature. On evaporation, the residue consists of ammonium bromide and phenylalanine. On extracting with boiling alcohol, the amino-acid remains undissolved. One crystallisation from hot water yields a pure product. The yield is about 4 grams. (Fischer.)

EXPT. 38.—**Synthesis of Phenylalanine from Benzaldehyde and Hippuric acid.**—Heat on the water-bath 16·5 grams of hippuric acid, 8·2 grams of fused sodium acetate, 10·6 grams of benzaldehyde, and 31 grams of acetic anhydride. The thick mass turns yellow and partly liquefies before again becoming semi-solid. Heat for three-quarters of an hour, cool, wash with a little cold water and filter; then rub with alcohol and again filter. Transfer to a basin, heat with a little hot water to remove sodium acetate, and filter and wash at the pump with hot water. The yield is 16 grams; m.p. 166–7°. This is the lactimide of benzoylaminocinnamic acid



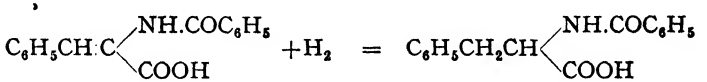
Lactimide of benzoylaminocinnamic acid.

To convert it into the acid, suspend 15 grams of the lactimide in 1500 c.c. of water, add 3 grams of sodium hydroxide in 30 c.c. of water, and heat on the water-bath until the whole of the substance has dissolved. The process may last an hour or more. Acidify the *hot* solution with hydrochloric acid, which precipitates the benzylaminocinnamic acid in colourless prisms, which, after recrystallisation from alcohol, melt slowly at about 210°. The yield is 14 grams. To reduce the acid, suspend the finely powdered substance in ten times its weight of water and add gradually rather more than the calculated amount of 2 per cent. sodium amalgam (1 gram of acid = 9 grams of amalgam). When the amalgam has completely decomposed, filter from the mercury, heat the reduced product, which is in solution as the sodium salt, on the water-bath, and fractionally precipitate with hydrochloric acid in approximately three fractions. The first fraction, which contains the bulk of the reduced acid, melts below 180°. The fraction of

higher melting point contains more of the unreduced acid and may be further heated with sodium amalgam; but an excess should be avoided, as uncrystallisable by-products are formed.

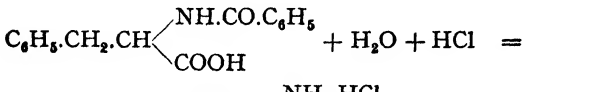
The reduced acid is separated from the unreduced portion by converting the latter into the lactimide with acetic anhydride, the lactimide being insoluble in sodium carbonate.

Dissolve the dry, powdered product in a little acetic anhydride by heating on the water-bath. On cooling and adding water, the acetic anhydride goes slowly into solution and the yellow lactimide, mixed with the colourless reduced acid, solidifies and is filtered and washed with water. Warm the precipitate on the water-bath with sodium carbonate solution. The acid dissolves completely, leaving the lactimide. Filter and precipitate the filtrate with hydrochloric acid. Filter and wash well with cold water to remove the acid and crystallise from alcohol. The yield from 10 grams is about 6 grams of pure acid; m.p. 184–5° (corr. 186–7°).



Benzoylaminocinnamic acid. Benzoylaminophenylpropionic acid.

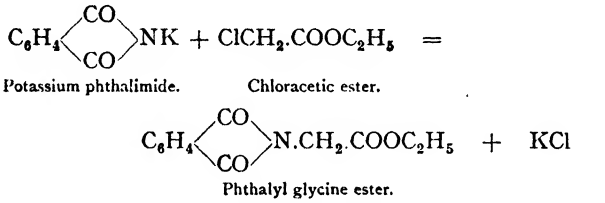
To hydrolyse the benzylaminopropionic acid, boil with 125 times its weight of 10 per cent. hydrochloric acid for eight hours, when the organic acid passes gradually into solution. Some benzoic acid crystallises on cooling and is removed by filtration. The filtrate is concentrated and the remainder of the benzoic acid extracted with ether. The solution is then evaporated to dryness on the water-bath. The residue is phenylalanine hydrochloride. To separate the phenylalanine, dissolve the dry hydrochloride in a little ammonia, and drive off the excess of ammonia on the water-bath. On concentrating the solution, the phenylalanine crystallises, on standing, in colourless plates. The yield is nearly theoretical (Erlenmeyer).



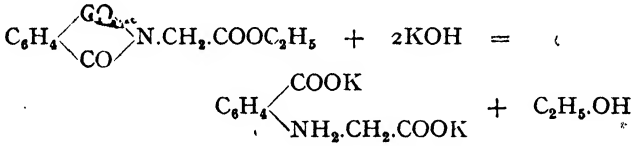
Phenylalanine hydrochloride. Benzoic acid.

EXPT. 39.—**Preparation of Glycine from Potassium phthalimide and Chloracetic ester.**—Heat together in the oil-bath at 150°

and with reflux condenser 10 grams of potassium phthalimide[1] and 6·5 grams of chloracetic ester, and stir occasionally with a glass rod to thoroughly mix the material. After half an hour the mass becomes pasty, and on cooling is dissolved in hot 50 per cent. alcohol. Filter the product, when cold, and wash first with cold 50 per cent. alcohol and then with water to remove potassium chloride. The yield is 6–8 grams. After crystallisation from dilute alcohol, it melts at 112–113°.



In order to hydrolyse the phthalyl glycine ester, 2·5 grams are boiled with reflux for a short time with 1·2 grams of potassium hydroxide in 12 c.c. of water. The ester passes into solution. Cool and add 2·2 c.c. of conc. hydrochloric acid. On standing a short time, colourless crystals of glycine phthalic acid separate. Filter and wash with ice-cold water until the filtrate no longer gives the reaction for chlorine.



Add now double the weight of 20 per cent. hydrochloric acid and boil in a reflux condenser with occasional shaking until a clear solution is obtained. The phthalic acid separates after two hours. Cool, dilute, and evaporate the filtrate on the water-bath. Add a little cold water, filter from phthalic acid,

[1] Phthalimide is prepared by passing dry ammonia gas through melted phthalic anhydride until it is saturated and gives the correct melting point (225°). Crystallise from acetic acid. The potassium salt is prepared by dissolving 10 parts of the powdered phthalimide in thirty times its weight of spirit, and adding 7 parts of potassium hydroxide in 40 c.c. of alcohol and cooling. Wash with alcohol and dry over sulphuric acid in a desiccator.

and evaporate the filtrate. The glycine, which remains, is washed with a little alcohol and dried (Gabriel).

$$C_6H_4\begin{cases}COOH\\NH.CH_2.COOH\end{cases} + H_2O =$$

Glycine phthalic acid.

$$C_6H_4(COOH)_2 + NH_2.CH_2.COOH$$

Phthalic acid Glycine.

EXPT. 40.—**Preparation of Synthetic Leucine from Isovaleric aldehyde.**—Mix together in a round flask (2 litres) attached to a reflux condenser 100 grams of potassium dichromate, 1 litre of water, and 130 grams of conc. sulphuric acid, warm to 90° on the water-bath and drop in gradually 80 grams of isoamyl alcohol. The product is then distilled, and the isovaleric aldehyde shaken in a tap-funnel with dilute sodium hydroxide solution, and, after removing the alkaline solution, with conc. sodium bisulphite solution. The crystalline mass is filtered and pressed and then distilled with sodium carbonate solution, whereby the valeric aldehyde distils. Add to the valeric aldehyde strong ammonia and shake well. Valeric aldehyde-ammonia separates in crystals. These are filtered and washed with water, and about 30 grams of the crystals are suspended in about their own weight of water, cooled and 14 c.c. of 50 per cent. hydrocyanic acid [1] gradually added. After standing for eight hours, with frequent shaking, add a mixture of 160 c.c. of conc. hydrochloric acid and 80 c.c. of water, whereby a precipitate is formed. On long boiling, the precipitate dissolves. Add a further 80 c.c. of water and boil again. Evaporate on the water-bath to remove hydrochloric acid and add 25 c.c. of water. Saturate with ammonia and after cooling filter the leucine and wash with cold water to dissolve out the ammonium chloride. The yield is about 10 grams. To remove colouring matter it may be dissolved in a large amount of hot water, boiled with animal charcoal, and filtered.

Polypeptides.—The fact that the proteins are hydrolysed by acids and alkalis to amino-acids appears to indicate that the

[1] This is prepared by distilling, in a good draught chamber and with great caution, 100 grams of coarsely powdered potassium ferrocyanide with a mixture of 40 c.c. conc. sulphuric acid and 140 c.c. water. A long, well-cooled condenser should be used, the end of which is attached air-tight to a receiver with a double tubulus, the second tubulus being provided with a long glass tube reaching to the flue opening. The receiver should be placed in ice. About 15 c.c. of hydrocyanic acid which distils will be of approximately 50 per cent. strength.

latter are united in the form of amides in which the carboxyl group of one amino-acid is linked to the amino-group of another. This view is supported by the fact that nitrous acid evolves little free nitrogen, that is to say, the polypeptides contain few if any amino-groups, and that they give the biuret reaction—a reaction peculiar to substances which contain linked amide groupings. Consequently, attempts have been made to combine the amino-acids of proteins to form chains which, according to the number of amino-acid groups present, are distinguished by the names di-, tri-, and tetra-peptides, or generally **polypeptides.**

For example, the dipeptide glycyl-glycine has been prepared in the following way: chloracetyl chloride combines with glycine to form chloracetyl glycine, and the latter with ammonia yields glycyl-glycine.

$$CH_2Cl.COCl + H_2N.CH_2.COOH \rightarrow CH_2Cl.CONH.CH_2.COOH$$
$$\rightarrow \underbrace{H_2N.CH_2.CO.NH.CH_2.COOH}_{\text{Glycyl-glycine.}}$$

The glycyl-glycine may be again combined with another halogen acid chloride, and the product again treated with ammonia and a tripeptide obtained:

$$\underbrace{CH_2Cl.CO.NH.CH_2.CO.NH.CH_2.COOH.}$$

or before decomposing the chloracyl derivative with ammonia the compound may be converted by the action of phosphorus pentachloride on the carboxyl group into the acyl chloride, and is thus in a condition to combine at the carboxyl end with another molecule of amino-acid. Thus, the above compound may be joined to a fourth molecule of glycine which, by a final treatment with ammonia, is converted into a tetrapeptide:

$$CH_2Cl.CO\,NH.CH_2\,CO.NH\,CH_2.COCl + NH_2.CH_2.COOH \rightarrow$$
$$CH_2Cl.CO.NH.CH_2.CO.NH.CH_2.CO.NH.CH_2.COOH \rightarrow$$
$$\underbrace{NH_2.CH_2.CO.NH.CH_2.CO.NH.CH_2.CO.NH.CH_2.COOH}_{\text{Tetrapeptide.}}$$

Various amino-acids have been strung together by adding groups at both ends of the chain, and it will be readily understood

that a large variety of polypeptides can be obtained in this way. Moreover, when it is considered that many of the amino-acids obtained from proteins contain asymmetric carbon and exist in optically active forms, the number of possible combinations is greatly increased. Thus, leucine, serine, cysteine, phenylalanine, tyrosine, etc., from proteins are lævorotatory, whereas alanine, valine, isoleucine, ornithine, lysine, arginine, etc., are dextrorotatory.

As a class, the polypeptides show a close resemblance to the simpler proteins (peptones). The majority are soluble in water; with the exception of some of the di- and tri-peptides they give the biuret reaction; they have a bitter, peptone-like taste and are readily hydrolysed by acids and in many cases by trypsin (see p. 102). The closest resemblance to the natural peptones is found among those polypeptides which have a long chain composed of a variety of amino-acid radicals.

That such polypeptides form part of the protein molecule is further confirmed by the isolation of certain members of this class of compounds. By graduated hydrolysis through the combined action of enzymes and chemical reagents, Fischer and Abderhalden obtained a tetrapeptide consisting of two molecules of glycine, one of *l*-tyrosine, and one of *d*-alanine from silk; Levene and Beatty isolated from gelatine by tryptic digestion glycylproline, a compound of phenylalanine and proline was obtained by Osborne and Clapp from gliadin (p. 93), and by partial hydrolysis of edestin from cotton-seed (p. 94) several di- and tri-peptides containing glutamic acid, tryptophane, leucine, glycine, etc., were prepared by Abderhalden. So far, however, with the exception of the dipeptide of Osborne and Clapp, none of the natural polypeptides have been synthesised.

Classification of the Proteins.—The proteins are classified as follows:

1. Protamines.
2. Histones.
3. Albumins and globulins.
4. Glutelins and gliadins.
5. Conjugated proteins (nucleoproteins, chromoproteins, glucoproteins).
6. Phosphoproteins.
7. Scleroproteins.
8. Derived proteins (metaproteins, proteoses, peptones, polypeptides).

Derived Proteins.—The simplest of these substances are the polypeptides, which have already been described. The **meta-proteins** include such substances as the acid and alkali albumins which are formed by the action of acids and alkalis on certain proteins; but little is known of their structure. The **proteoses** and **peptones** are commonly prepared by the peptic digestion of proteins. Ammonium sulphate precipitates the proteoses, whilst the peptones are obtained from the filtrate by adding alcohol after removing ammonium sulphate. Both groups of substances are readily soluble in water and are not coagulated by heat.

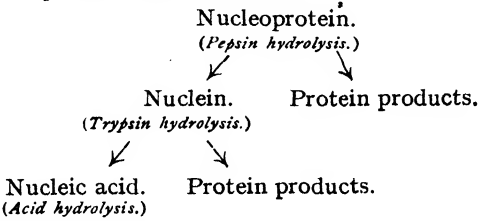
The **scleroproteins** include a number of substances such as **gelatin** from skin and cartilage, **keratin** from hair, hoof, horns, and nails; **elastin** from connective-tissue, **sericin** from silk, and **spongin** from sponges, all of which, though closely allied to the typical proteins, cannot be classified under any of the other groups.

The **phosphoproteins** are proteins which, as their name indicates, contain a certain proportion of phosphorus (0·5–1·5 per cent.). Unlike the nucleoproteins (see below), which also contain phosphorus, they yield no pyrimidine nor purine bases on hydrolysis. The most important members of the group are **casein** of milk and **vitellin**, found in egg-yolk. They are acid substances, insoluble in water, but readily soluble in alkalis, forming definite salts.

EXPT 41.—**Preparation of Casein and Lactose from Milk.**—Dilute 250 c.c. of fresh milk with 1 litre of distilled water, and add so much acetic acid that the solution contains 0·1 per cent. of acid (1·25 grams). Wash the precipitate two or three times by decanting rapidly. Rub the precipitate in a mortar with the least possible quantity required for solution of dilute sodium hydroxide solution (0·1 per cent.), taking care that the final solution is neutral. Keep the washings. Filter through a cloth until the liquid is only faintly opalescent. The filtrate is again acidified with acetic acid as before and the precipitate again washed, and the process of solution and precipitation repeated. It is drained, made into a paste with 97 per cent. alcohol to remove water, filtered and washed with pure alcohol, and then with ether to remove fat, and dried in air or over sulphuric acid in a vacuum desiccator. It is a white, amorphous powder, soluble in the hydroxides, carbonates, and phosphates of the alkalis, and also in lime and baryta water. The yield is about 6 grams.

The lactose is prepared from the filtrate from the casein. Boil the liquid to precipitate the soluble proteins, filter, neutralise with magnesium carbonate, and evaporate on the water-bath; extract with alcohol, dissolve the residue in water, filter, and concentrate to a syrup. On standing, crystals of lactose separate It may be recrystallised from hot water. Prepare lactosazone as described in Vol. I, p. 151. It forms characteristic nests of hairy crystals which take much longer to separate than the glucosazone. It does not, like glucose, reduce Barfoed's reagent (1 part of cupric acetate in 15 parts of water; to 200 c.c. of this solution 5 c.c. of 38 per cent. acetic acid are added), but reduces Fehling solution and ammonia-silver nitrate solution (see Vol. I, p. 160). On oxidation with nitric acid it forms **mucic acid.** Evaporate 10 grams of lactose with 100 c.c. of nitric acid of sp. gr. 1·15 in a basin to 20 c.c., when a pasty mass of mucic acid crystals separates. Dilute with cold water, filter, and wash with a little water.

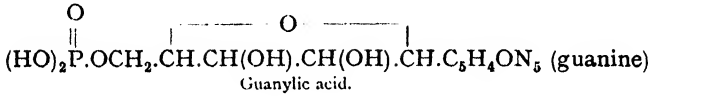
The **Conjugated proteins** are complex substances which can be resolved by the action of enzymes or acids into a new and simpler protein on the one hand, and, on the other, into a second substance of varying character, known as the **prosthetic group.** They are divided into nucleoproteins, chromoproteins, and glucoproteins. The simplest nucleoproteins are found in fish spermatozoa combined with protamines (p. 94), and have been prepared from the thymus gland, the pancreas, and yeast-cells. When hydrolysed by pepsin, a portion of the protein is removed, leaving **nuclein,** which on further hydrolysis by trypsin yields an additional amount of protein and nucleic acid.



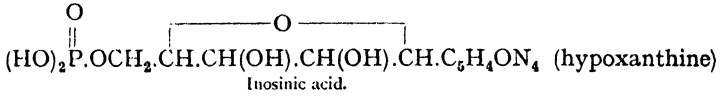
If the nucleic acids are further broken up by acids they yield a variety of products, among which phosphoric acid, carbohydrates, as well as pyrimidine and purine bases, have been found. Among the pyrimidine bases, thymine, cytosine, and uracil have been identified (p. 59), whilst the purine bases include adenine,

guanine, and hypoxanthine. The carbohydrate has been identified in yeast nucleic acids as *d*-ribose; in thymus nucleic acid it is a hexose. A simpler form of nucleic acid, which is found in certain organs, yields, on hydrolysis, phosphoric acid, ribose, and guanine, and has been named **guanylic acid**, and another, known as **inosinic acid**, breaks up into the first two substances and hypoxanthine.

The structure of these and the more complex nucleic acids has been very fully discussed by Levene and Jacobs, who describe the simplest nucleic acid, composed of one molecule each of acid, base, and sugar as a mono-nucleotide. Guanylic and inosinic acids belong to this class.



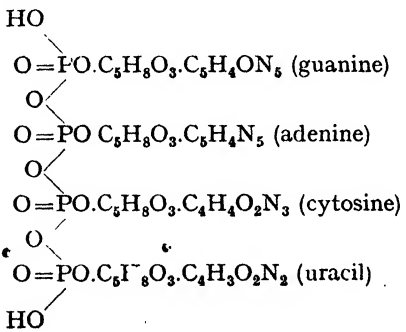
Guanylic acid.



Inosinic acid.

After splitting off phosphoric acid the compound of sugar and base which remains is called a **nucleoside. Guanosin** is guanine *d*-riboside, and **adenosin** is adenine *d*-riboside. A guanine-hexoside has been obtained by Levene from thymus nucleic acid.

The more complex nucleic acid derived from yeast is regarded as a combination of four nucleotides (tetranucleotide) in which two pyrimidine and two xanthine bases are present.



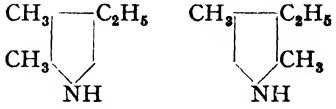
Yeast nucleic acid.

The Chromoproteins.—The most important member of this group is **hæmoglobin,** the red colouring matter of vertebrate blood to which the green chlorophyll of plants is related. Hæmoglobin has the peculiar property of combining directly with oxygen to form **oxyhæmoglobin,** which like hæmoglobin can be readily crystallised and so obtained in a state of purity. They contain iron as an essential constituent, and, assuming that one atom only is present, the formula for hæmoglobin will be represented by

$$C_{758}H_{1203}O_{195}N_{213}FeS_3.$$

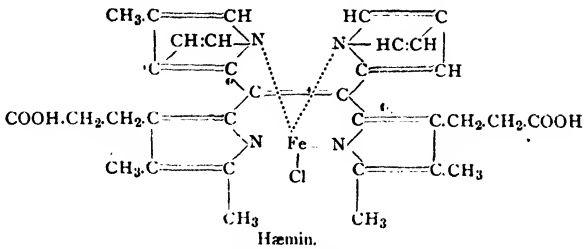
Weak acids readily hydrolyse oxyhæmoglobin, yielding the protein, **globin** and the prosthetic group, **hæmatin.** Closely related to hæmatin is **hæmin** which can be directly obtained from blood by warming the latter with glacial acetic acid and a trace of common salt. The dark coloured plates which crystallise are hæmin, and are formed by the action of hydrochloric acid on hæmatin. Hæmin, $C_{33}H_{32}O_4N_4FeCl$, loses its atom of iron on treatment with hydrogen bromide and yields **hæmatoporphyrin,** $C_{33}H_{38}O_6N_4$, which, by the successive action of different reagents, passes through a series of changes yielding **aetioporphyrin,** $C_{31}H_{36}N_4$, a product identical with one of the cleavage products of chlorophyll, obtained from aetiophyllin, $C_{31}H_{34}N_4Mg$.

Both hæmin and aetiophyllin may be regarded as derived from one and the same parent substance, namely, dimethyl ethyl pyrrole, or from a mixture of isomeric compounds possessing the following formulæ:

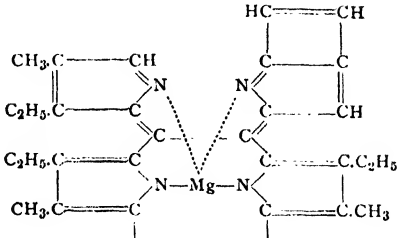


Dimethyl ethyl pyrroles.

Four of these are united in the molecule of hæmin and chlorophyll, but the atom of iron in hæmin is replaced by one of magnesium in chlorophyll. The following formulæ have been allotted to hæmin and aetiophyllin:



Hæmin.
$C_{33}H_{32}O_4N_4FeCl$



Aetiophyllin.
$C_{31}H_{34}N_4Mg$

Closely related to the colouring matter of the blood are the bile pigments **bilirubin** and **biliverdin**, the structure of which is still undisclosed.

The Glucoproteins.—It has long been known that certain proteins yield, on hydrolysis, substances giving the reactions of carbohydrates. Egg-albumin, for example, gives a reducing sugar, on hydrolysis. The more important of the glucoproteins are the **mucins** and **mucoids**. The former are viscid substances which are secreted by the epithelial cells of animals, and are very similar to the mucoids, which, however, are not precipitated from alkaline solution by acetic acid. From mucine, **glucosamine** has been isolated, which has been identified as amino-glucose from its reactions, its conversion into glucose, and by its synthesis.

$CH_2OH.CH(OH).CH(OH).CH(OH).CH(NH_2).CHO.$
Glucosamine.

It may be regarded, therefore, as intermediate between the carbohydrates and the amino-acids, and is probably a secondary

product derived by hydrolysis from a more complicated carbohydrate group in the protein molecule. Glucosamine is readily obtained by the action of conc. hydrochloric acid on **chitin**, the hard shell of crustacea, beetles, etc.

EXPT. 42.—**Preparation of Glucosamine hydrochloride.**—Crab or lobster shell is carefully freed from soft tissue and soaked in dilute hydrochloric acid for twenty-four hours. The shell becomes soft and can be easily cut with scissors into small pieces. Place about 100 grams in a basin, cover with conc. hydrochloric acid, and boil gently on a sand-bath. Most of the shell rapidly dissolves and gives a brown solution. Dilute with a little water, filter from undissolved substance and evaporate the filtrate on the water-bath until crystals appear on the surface. Filter, wash with a little alcohol and, if necessary, recrystallise from hot water with the addition of a little charcoal. It forms highly refractive, colourless crystals which reduce Fehling's solution, form ordinary glucosazone and are dextrorotatory.

The glutelins and gliadins have a vegetable origin and are present in many kinds of seeds. The **glutelins** are insoluble in neutral aqueous solutions, in salt solutions and in alcohol. They yield, on hydrolysis, 12–20 per cent. of glutamic acid and 5–10 per cent. of arginine and leucine. The **gliadins** (gliadin, hordein, and zein) are soluble in alcohol, but not in water, though their salts with acids and alkalis dissolve. They are present in the seeds of all cereals. **Gliadin**, from the gluten of wheat and rye, and **hordein**, from barley, contain more glutamic acid and less arginine than the glutelins. **Zein** is obtained from maize and contains more leucine and less glutamic acid than the other gliadins.

The albumins and globulins are coagulable proteins forming the more important constituents of the majority of animal and vegetable tissues. They contain sulphur, little or no phosphorus, and, with the exception of a carbohydrate group, no other prosthetic group.

A general method for their separation is by precipitation with ammonium or magnesium sulphate, the salt being then removed by dialysis. From the dialysed solution, alcohol, in which they are insoluble, precipitates the proteins.

Albumins are soluble in water and precipitated only from solutions saturated with ammonium sulphate, but not by magnesium sulphate, whereas the globulins, though soluble in very dilute salt solutions, are thrown down by magnesium sulphate before saturation is reached. In this way the two groups may be differentiated. As a small amount of salt is necessary to retain the globulins in solution, when, on dialysis, the salt is removed beyond a certain point, precipitation occurs.

Of the albumins, **egg-** and **serum-albumin** are the most important and both have been prepared in microscopic crystals by slow separation from ammonium sulphate solution. Of the animal globulins, **serum-globulin** of the blood is the best known. There are numerous vegetable globulins, such as **edestin** from hemp and other seeds which have been prepared in the crystalline form. In none of these groups do the products of hydrolysis differ greatly, as may be seen from the table on p. 95.

The Protamines and Histones.—The **protamines** comprise a small number of proteins of strongly basic character which occur in combination with nucleic acids (p. 89) in the form of nucleoproteins. They were first investigated by Miescher and later by Kossel. They are obtained from the ripe spermatozoa of fish by extraction with dilute sulphuric acid and precipitating the filtered solution with alcohol. Their solutions are not coagulated on boiling, but they respond to many of the usual protein reactions. Characteristic of these substances is the large proportion of arginine, which enters into their composition and which may in some cases reach 88–89 per cent.

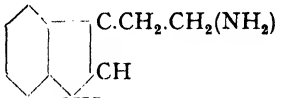
The **histones** are also basic substances with a high percentage of nitrogen (17–20 per cent.). Though the group is not very clearly defined they stand midway between the protamines and the coagulable proteins and proteoses. It will be seen from the table that they yield a much smaller amount of arginine.

Bacterial decomposition of Amino-acids.—Protein substances and the amino-acids derived from them, when submitted to the action of putrefying (anaerobic) bacteria, break down generally with the loss of carbon dioxide. Valine yields isobutylamine;

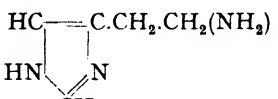
Hydrolytic Products of the Proteins.

——	Keratin from Horn.	Caseinogen (cow).	Hæmoglobin.	Gliadin (wheat).	Edestin (hemp).	Serum-globulin.	Egg-albumin.	Thymus histone.	Salmine.
Glycine . . .	0·3	—	0	0·02	3·8	3·5	—	—	—
Alanine . . .	1·2	0·9	4·19	2·0	3·6	2·2	2·2	—	—
Valine . . .	5·7	1·0	—	0·2	+	+	2·5	—	4·3
Leucine and Isoleucine.	18·3	10·5	30·0	5·61	20·9	18·7	10·7	—	—
Aspartic acid .	2·5	1·2	4·43	0·58	4·25	2·5	2·2	—	—
Glutamic acid .	3·0	11·0	1·73	37·33	6·3	8·5	9·1	3·6	—
Serine . . .	0·7	0·23	0·56	0·13	0·33	—	—	—	7·8
Cystine . . .	7·0	+	0·31	0·45	0·25	0·7	—	—	—
Lysine . . .	+	6·0	4·28	—	1·0	—	3·7	7·7	—
Arginine. . .	2·3	4·8	5·42	3·16	11·7	—	4·9	14·3	87·4
Proline . . .	3·6	3·1	2·34	7·06	4·1	—	3·5	—	11·0
Oxyproline . .	—	0·3	1·04	—	2·0	—	—	—	+
Histidine . .	—	2·6	10·96	0·61	1·1	—	1·7	1·2	—
Tryptophane .	—	1·5	+	+	+	+	+	—	—
Phenylalanine .	3·0	3·2	4·24	2·35	3·1	3·8	5·0	—	—
Tyrosine. . .	4·6	4·5	1·53	1·2	2·11	2·5	1·7	6·3	—

leucine gives isoamylamine; tetra- and penta-methylene diamine are obtained from ornithine and lysine respectively; phenylalanine yields phenylethylamine and tyrosine the hydroxy-derivative, whilst tryptophane forms indole ethylamine, and histidine, iminazole ethylamine.



Indole ethylamine.



Iminazole ethylamine.

In other cases reduction takes place, ammonia being given off and the corresponding saturated fatty acid formed. Phenylalanine under certain conditions gives phenylpropionic acid.

$$\underset{\text{Phenylalanine.}}{C_6H_5.CH_2.CH(NH_2).COOH} + H_2 = \underset{\text{Phenylpropionic acid.}}{C_6H_5.CH_2.CH_2.COOH} + NH_3$$

On the other hand, aerobic bacteria oxidise the amino-acid a fatty acid containing one carbon atom less, both ammonia arbon dioxide being removed. Leucine yields isovaleric

$$\underset{\text{Leucine.}}{(CH_3)_2.CH.CH_2.CH(NH_2).COOH} + O_2 =$$

$$\underset{\text{Isovaleric acid.}}{CH_3)_2.CH.CH_2.COOH} + NH_3 + CO_2.$$

g to Ehrlich, convert the amino-acids into oxide, and water. Leucine forms isoamyl

$$\underset{\text{Leucine.}}{CH_2.CH(NH_2).COOH} + H_2O =$$

$$H_2.CH_2.OH + CO_2 + NH_3.$$

lcohol.

ff.—It has been conclusively estabestion the protein molecule is comsynthesis must take place within the blood before and during able to prove that it is in the

form of amino-acids that protein synthesis begins. For it is found that the more complex polypeptides introduced into the blood stream are excreted unchanged, and that it is only the amino-acids which are utilised and disappear.

The series of amino-acids which are essential to nutrition has been the subject of prolonged and careful investigation. Hopkins fed rats on a dietary of known amino-acids in certain proportions. When the amino-acid mixture comprised the whole assembly contained in typical protein, growth was always maintained, but if hydrolysed casein (in which the tryptophane molecule is absent) was administered, loss of weight ensues and the animal dies. It appears as if the organism was incapable of synthesising the indole ring. In the same way, if arginine and histidine are removed, loss of weight occurs, but is restored if these substances are replaced. Glutamic and aspartic acids produce no such effect, and it may therefore be that the necessary machinery is present to effect their synthesis. Tyrosine can also be dispensed with, for gelatin which contains none can, with the addition of tryptophane, sustain life.

Although the absence of histidine and arginine cause decrease in weight, if either is present the curve of body-weight rises. That the two should be apparently interchangeable seems not improbable from a comparison of their closely related structure (see pp. 71, 72).

Osborne and his collaborators have carried out similar experiments with vegetable proteins and, like Hopkins, find that proteins deficient in tryptophane diminish the animal weight, whereas most of the other vegetable proteins produce an increase.

Seeing that such very different proteins as gliadin, edestin, and casein are deficient in either lysine, glycine, phosphoproteins, or purines, it is clear that the animal organism must be capable of synthesising these complex structures.

The following table gives a survey of the protein content and other constituents of various food-stuffs.

Average Food Analyses.

Food-stuff.	Protein.	Fat.	Carbohydrate.
Meat . . .	14·5	16·1	—
Fish . . .	10·9	2·4	—
Milk . . .	3·3	4·0	5·0
Butter . .	1·0	83·0	—
Flour. . .	11·4	1·0	75·0
Rice . . .	7·4	0·4	79·2
Peas . . .	22·6	1·7	53·2
Potatoes. .	1·8	0·1	14·7
Fruit. . .	0·4	0·5	8·0

CHAPTER VII

FERMENTATION AND ENZYME ACTION

FERMENTATION may be broadly described as a process by which certain products are elaborated from organic substances as the result of the activity of living cells. The lifeless products of the cells which directly induce these changes are termed *enzymes* (ἐν ζύμῃ, in yeast) and act either in the presence or absence of the living organism. Thus, the original meaning of the word fermentation, which was derived from *fervere*, to boil, and which connected it with the appearance of frothing, owing to the evolution of gas, has entirely disappeared.

Our knowledge of the dependence of alcoholic fermentation on the presence of the living yeast-cell is due to Pasteur, who was able to prove at the same time that the lactic and butyric fermentations were kindred phenomena. Not only so, but he showed that many diseases had their origin in the existence of lower organisms which effected active chemical changes in the living animal and plant, and in this way laid the foundation of bacteriology and biochemistry. Until recently a more or less sharp line of demarcation was drawn between a change, such as fermentation by yeast, and those reactions which are brought about by substances to which the name **enzyme** has been given. Liebig and Wöhler showed that emulsin, the enzyme of bitter almonds, occurred along with the glucoside, amygdalin, and that when the cell of the almond was ruptured in the presence of water the two substances were brought into contact, with the result that the glucoside was resolved into benzaldehyde, hydrocyanic acid, and glucose.

By suitable methods it is possible to separate the enzyme

from the glucoside and from the cells of the almond, and the reaction may consequently be carried out under conditions which preclude the possibility of any living material being concerned in the change. A similar reaction had been observed by Kirchhoff, who discovered the action of diastase on starch (Vol. I, p. 23). Ferment actions were therefore divided into those induced by organised ferments, such as yeast, and by unorganised ferments, such as emulsin and diastase. This distinction has now disappeared owing to the discovery by E. Buchner in 1896 that the juice expressed from the ruptured yeast-cell can set up fermentation in sugar solutions, and is therefore independent of the presence of the living organism. Enzymes may therefore be regarded as the " chemical reagents " of the living organism whereby disintegration and synthesis of the most diverse kinds are accomplished.

Catalytic Action of Enzymes.—The general view that catalysis determines an increase in the velocity of a reaction (which normally proceeds at a definite, though extremely slow, rate in the absence of the foreign substance or catalyst) is quite in harmony with all that is known of enzymes. Moreover, like most catalytic processes, the action is reversible, that is, the same equilibrium point is reached when either the original substance or its products are brought within its sphere of action (see p. 103). The speed of formation increases with the amount of enzyme, whilst the enzyme does not appear in the product nor does the quantity taking part in the reaction bear any definite molecular relation to the substance acted on. All these are the usual characteristics of a catalyst. In one respect does an enzyme differ notably from an inorganic catalyst, such as the finely divided metals or mineral acids, namely, in the *specific* nature of its action already briefly referred to in connection with the α- and β-glucosides (p. 42), and considered in greater detail on p. 101.

Chemical Action of Enzymes.—The majority of enzyme reactions are of a simple hydrolytic character and the energy changes are small (*e.g.*, the saponification of fat, the conversion of starch into sugars, the liberation of sugars from glucosides), and most of them can be carried out equally well with inorganic catalysts such as acids or alkalis. Even some of the more

complicated enzyme actions, such as the oxidation of alcohol to acetic acid, may be effected by finely divided metals (platinum).'

There is a large amount of indirect evidence in support of the view that an enzyme enters into definite combination with the substance which undergoes change or, as it is usually termed, **substrate.** This combination is attributed to its colloidal nature. The properties of colloids depend mainly on the large surface of the particles in proportion to their size. It has been shown that, if a dissolved substance lowers the surface tension of a liquid, there is a tendency for the substance to accumulate at the interface between solid and solution. It can be readily understood that such a process must occur when a solution is in contact with colloidal substances, whereby the latter tend to withdraw the substance from solution. The phenomenon is called **adsorption,** and it will occur whenever the surface energy of a solution, from whatever cause, is lowered.

In this way the substrate becomes attached to the enzyme which is assumed to be the seat of the chemical change. Of the enzymes themselves extremely little is known; for this very property of adsorption, together with their amorphous character, renders them difficult to purify. Some appear to resemble the proteins (p. 70), others again contain little nitrogen. In many cases the enzyme seems to conform in composition to the substance it hydrolyses. Many of them are most active in neutral solution at about blood temperature (37°), others act best in a faintly acid liquid, whilst trypsin works well in a faintly alkaline solution (p. 77). In aqueous solution they are almost all destroyed at temperatures above 70°, though in absence of water they are more stable and may be heated to 100° or even higher without losing their activity. In order to follow the action of an enzyme apart from any living organism with which it may be contaminated, an addition of chloroform, toluene, or sodium fluoride is made, which, whilst killing bacterial life, does not affect the enzyme.

Specific Action of Enzymes.—Among the more familiar hydrolytic enzymes which attack carbohydrates are **diastase** which converts starch and glycogen into maltose; **maltase,** which con-

verts maltose into glucose; and **invertase**, which breaks up cane-sugar into fructose and glucose. Lactase, which is found in **kephir grains**—nodules producing a fermented milk known as koumiss—hydrolyses milk-sugar to glucose and galactose. Among the glucoside enzymes are **emulsin** of bitter almonds, which hydrolyses amygdalin, and **myrosin** from black mustard seed which decomposes sinigrin into allyl thiocyanate, $C_3H_5N{:}CS$, potassium hydrogen sulphate, and glucose. Emulsin, moreover, hydrolyses the following natural glucosides.

Glucoside.	Products.
Salicin .	Saligenin, $C_6H_4\begin{cases}OH\\CH_2.OH\end{cases}$ + glucose.
Helicin .	Salicylaldehyde, $C_6H_4\begin{cases}OH\\CHO\end{cases}$ + glucose.
Arbutin .	Quinol, $HO.C_6H_4.OH$ + glucose.
Coniferin .	Coniferyl alcohol, $\begin{matrix}OH\\CH_3O\end{matrix}\!\!>C_6H_3.CH{:}CH.CH_2.OH$ + glucose

In addition to the above are a number of proteolytic enzymes (hydrolysing proteins), among which the following may be mentioned: **trypsin** of the pancreas and **erepsin** of the intestinal tract, which convert proteins into amino-acids; **pepsin** of the gastric juice, which breaks them down into simpler proteins (proteoses and peptones, p. 88); **arginase, guanase**, and **adenase**, present in the liver and other organs, which hydrolyse arginine (p. 71), guanine (p. 62), and adenine (p. 62) respectively, the first giving ornithine and urea:

$$\begin{matrix}C(:NH)\cdot NH_2\\|\\NH(CH_2)_3.CH(NH_2).CO_2H\\\text{Arginine.}\end{matrix} \rightarrow CO(NH_2)_2 + H_2N(CH_2)_3.CH(NH_2).CO_2H$$

Urea. Ornithine.

the second xanthine and the third hypoxanthine (p. 61).

Further, **lipase**, present in the pancreas and liver, and in oily seeds, hydrolyses fats and esters, and **urease** converts urea into ammonium carbonate and is responsible for the ammoniacal smell of stale urine.

$$OC\begin{matrix}NH_2\\NH_2\end{matrix} + 2H_2O = (NH_4)_2CO_3.$$

In addition to the above is a group of oxidising enzymes or **oxidases**, such as are present in *Bacillus aceti*, which converts ethyl alcohol into acetaldehyde and acetic acid, a few less well defined reducing enzymes or **reductases**, and acid-forming enzymes present in the lactic and butyric ferments, which produce lactic and butyric acids from various polyhydric alcohols and carbohydrates.

The table on p. 104 gives the name and source of the various enzymes referred to, the substrate, and the name of the product.

It should be remembered that in the above changes the action is specific, that is to say, each enzyme is restricted to certain substrates and to no others. The action is even more limited, for it is only certain optically active forms which are attacked. Among the polypeptides, to which reference is made on p. 86, trypsin hydrolyses one group of active compounds, but not the stereoisomeric forms, in the same way that yeast ferments only a few of the active monosaccharoses (p. 42).

Hydrolysed.	Not hydrolysed.
d-Alanyl-*d*-alanine	*d*-Alanyl-*l*-alanine.
d-Alanyl-*l*-leucine	*l*-Alanyl-*d*-alanine.
l-Leucyl-*l*-leucine	*d*-Leucyl-*l*-leucine.
l-Leucyl-*d*-glutamic acid	*l*-Leucyl-*d*-leucine.

Reversibility of Enzyme Action.—The reversibility of enzyme action has now been observed in a very large number of cases. Lipase, in addition to hydrolysing esters, has the property, under certain conditions, of effecting their synthesis, as well as that of butyrin from glycerol and butyric acid and of olein from glycerol and oleic acid. Emulsin and maltase, in presence of an alcohol and a monosaccharose, produce a variety of α- and β-alkyl saccharosides. Indications have also been obtained that trypsin,

Enzyme.	Substrate.	Product.	Source.
	Polysaccharoses.		
Diastase (Ptyalin)	Starch / Glycogen	Maltose	Germinating grain
	Disaccharoses.		
Maltase	Maltose	Glucose	Yeast, malt
Invertase	Cane-sugar	Fructose, glucose	Yeast extract
Lactase	Milk-sugar	Glucose, galactose	Kephir grains
	Glucosides.		
Emulsin	Amygdalin	Glucose, benzaldehyde and hydrocyanic acid	Bitter almonds
Myrosin	Sinigrin	Glucose, $KHSO_4$, allylthiocyanate	Mustard seed
	Proteins.		
Trypsin	Proteins	Amino-acids	Pancreas
Pepsin	,,	,,	Gastric secretion
Erepsin	Proteoses and peptones	,,	Intestine
Arginase	Arginine	Ornithine and urea	Liver and kidney
	Purine compounds.		
Guanase	Guanine	Xanthine	Spleen and liver
Adenase	Adenine	Hypoxanthine	,,
	Other Substances.		
Lipase	Fats and esters	Acid and alcohol	Pancreas, liver and seeds
Urease	Urea	Ammonium carbonate	Enzyme from micrococcus ureæ, soya beans, etc.

pepsin, and erepsin can synthesise proteins from the same amino-acids which they form on hydrolysis. Certain anomalous results have been observed in the case of yeast extract, which contains maltase but converts glucose into isomaltose instead of maltose, and kephir grains, which convert a mixture of glucose and galactose into isolactose in place of lactose; but no satisfactory explanation is so far forthcoming.

Alcoholic fermentation has been studied more extensively than perhaps any other biochemical process, yet, in spite of the labour which has been expended upon it, the chemical changes involved are still obscure. The decomposition of glucose on fermentation, which was originally represented by the simple equation,

$$C_6H_{12}O_6 = 2C_2H_6O + 2CO_2$$

has gradually resolved itself into a series of reactions of growing complexity. For, in addition to the higher alcohols included in the term **fusel oil**, Pasteur found glycerol and succinic acid. The alcohols of fusel oil and succinic acid have, however, been shown by Ehrlich to be derived from certain protein cleavage products of the yeast-cells (p. 96), and are not present if yeast juice is employed. Since the discovery of yeast juice, which contains an enzyme or enzymes capable of producing fermentation, the latter process has been brought within the domain of enzyme action. But although it has, in a sense, been simplified by this discovery, it has at the same time been complicated by the observations of Harden and Young, who showed that yeast juice may, by dialysis, be separated into two substances, an enzyme and a co-enzyme, which must act conjointly to bring about fermentation.

If we examine the formulæ of the four natural hexoses which undergo fermentation, using the aldehyde and ketone structure given on pp. 34, 36, it is clear that one part of the molecule is reduced at the expense of the other, the latter being thereby oxidised, or, in other words, that there is a transference of hydrogen in one direction and of oxygen in another to form alcohol and carbon dioxide.

$$\begin{array}{l} \mathrm{CHO} \\ | \\ \mathrm{CH.OH} \\ | \\ \mathrm{CH.OH} \\ | \\ \mathrm{CH.OH} \\ | \\ \mathrm{CH.OH} \\ | \\ \mathrm{CH_2.OH} \end{array} \quad = \quad \begin{array}{l} \mathrm{CO_2} \\ \\ \mathrm{CO_2} \\ \\ \mathrm{CH_2.OH} \\ | \\ \mathrm{CH_3} \\ \\ \mathrm{CH_2.OH} \\ | \\ \mathrm{CH_3} \end{array}$$

As most enzyme actions are hydrolytic, it seems only natural to suppose that the change is effected by the addition and removal of the elements of water. The breaking up of the sugar molecule in this manner is not, however, confined to the action of yeast, but has been imitated by Duclaux, who found that alcohol is formed by the action of alkali on sugar in presence of sunlight. With more dilute alkali, however, a large quantity of lactic acid is formed, and it has consequently been suggested that the precursor of alcohol is a substance giving rise to lactic acid; for it cannot be lactic acid itself, seeing that this substance is unattacked by yeast or yeast juice. There are many reasons for supposing that methyl glyoxal, $CH_3.CO.CHO$, is the compound in question.

On the one hand, methyl glyoxal in alkaline solution readily passes into lactic acid by addition of the elements of water, and, on the other, it is transformed in the animal organism into glucose. The link between glucose and methyl glyoxal may very well be glyceric aldehyde and dihydroxyacetone (from which fructose is obtained by synthesis, p. 34), for both these substances are fermentable by yeast.

$$\mathrm{CH_2.OH.(CH.OH)_4.CHO} \rightleftharpoons 2 \begin{cases} \underset{\text{Dihydroxyacetone.}}{\mathrm{CH_2.OH.CO.CH_2.OH}} & \overset{-\mathrm{H_2O}}{\underset{+\mathrm{H_2O}}{\rightleftharpoons}} \\ \underset{\text{Glyceric aldehyde.}}{\mathrm{CH_2.OH.CH.OH.CHO}} & \end{cases}$$

$$\underset{\text{Methyl glyoxal.}}{\mathrm{CH_3.CO.CHO}}.$$

Assuming the latter to pass into the tautomeric "enol" form and then to undergo hydrolysis, alcohol and carbon dioxide would result.

$$\mathrm{CH_3.C(OH):CO} + \mathrm{H_2O} = \mathrm{CH_3.CH_2.OH} + \mathrm{CO_2}.$$

Another theory put forward by Neuberg is that pyruvic acid is formed as an intermediate product which, under the action of a ferment, **carboxylase**, present in yeast, is broken up into acetaldehyde and carbon dioxide, the former then undergoing reduction by a reductase contained in yeast.

$$\underset{\text{Pyruvic acid.}}{CH_3.CO.COOH} = CH_3.CHO + CO_2$$

Neuberg has succeeded in confirming this view by adding sodium sulphite to the fermenting liquid when the acetaldehyde bisulphite compound was isolated in quantity corresponding with nearly three-quarters of the theoretical amount.

EXPT. 43.—**Preparation of Lactic acid from Cane-sugar and also from Milk. From Cane-sugar.**—Dissolve 50 grams of cane-sugar in 250 c.c. of water, add 50 c.c. of sour milk, 5 grams of St. Ivel [1] cheese, and 15 grams of zinc carbonate, and place in the incubator at 35° for eight to ten days, with occasional stirring. Filter the mixture through fine unstarched calico at the pump. Boil up the solid portion with 200 c.c. of water and filter hot, repeat the process with the undissolved residue, adding a further 100 c.c. of water, filter hot as before and add to the first filtrate. On cooling, a crystalline mass of colourless zinc lactate,

$$(C_3H_5O_3)_2Zn + 3H_2O,$$

separates. Evaporate the original filtrate with the mother liquor from the zinc lactate on the water-bath until crystals appear. Cool and filter. The zinc lactate obtained from this portion is usually coloured and should be re-crystallised. A further quantity may be obtained by again concentrating the mother liquors. The total yield is 25–30 grams.

Lactic acid may be obtained from the zinc salt by adding dilute sulphuric acid sufficient to combine with the zinc present, and shaking out with ether, in which the lactic acid dissolves. On distilling the ether on the water-bath the lactic acid remains as a colourless, viscid liquid.

From Milk.—To 500 c.c. of fresh milk, add 15 grams of St. Ivel cheese (or 30 c.c. of "starter"), and 20 grams of zinc carbonate, and incubate for eight to ten days at 35° with occasional stirring.

[1] Any other strongly flavoured cheese will do. In place of sour milk what is known in cheese-making as "starter," *i.e.*, curdled milk containing the pure lactic ferment, is more efficient.

Filter through calico as above. Boil the filtrate with a little zinc carbonate for a time, cool and filter. The filtrate will now be clear. Boil the solid residue (consisting of casein, fat, unchanged zinc carbonate and zinc lactate) with 300 c.c. of water for some time, cool and let stand so that the fat solidifies. Filter.

Concentrate the combined filtrates on the water-bath until crystallisation begins. Filter the crystals of zinc lactate. Concentrate the mother liquors, when a further quantity of the salt will separate. The total yield is about 20 grams, which, assuming that a milk-sugar yields two molecules of lactic acid, will represent about 50 per cent. of the theoretical amount.

The Action of Enzymes in Digestion.—The process of digestion is largely effected by enzyme action. Starch is hydrolysed by ptyalin (diastase) of the saliva. In the stomach the proteins are converted into proteoses and peptones by hydrochloric acid and the pepsin of the gastric juice, the secretion of which is stimulated by the presence of food. Thence the partially digested material passes into the small intestine, where the acid in the food causes the liberation of a definite chemical substance, **secretin**, which is absorbed into the blood-stream and stimulates an active secretion by the pancreas of **trypsinogen** and other digestive enzymes. At the same time, the mucous membrane of the intestine furnishes an enzyme, **enterokinase**, which has the property of converting trypsinogen into trypsin. Trypsinogen is therefore the precursor or " zymogen " of trypsin and enterokinase, a " ferment of ferments."

The secretion of pancreatic juice and, therefore, of trypsin is not a continuous process, but is regulated by this simple mechanism according to the needs of the organism. In the pancreas the final breakdown of the protein material into amino-acids takes place. The fate of the amino-acids has been discussed on pp. 30, 96.

CHAPTER VIII

THE ESSENTIAL OILS

The Essential Oils are distinguished from the **fixed oils** or glycerides of fatty acids described in Chapter II by their generally pleasant aroma and by the fact that they may be dis-

ESSENTIAL OILS

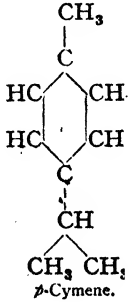
Name.	Source.	Chief constituents.
Anise oil	*Pimpinella anisum*	Anethole, estragol
Bergamot oil	*Citrus bergamea*	Linalyl acetate, linalol, *d*-limonene
Bitter almond oil	*Amygdalus communis*	Benzaldehyde
Caraway oil	*Carum carvi*	Carvone
Cinnamon oil	*Cinnamomum Zeylanicum*	Cinnamic aldehyde
Clove oil	*Eugenia caryophyllata*	Eugenol
Eucalyptus oil	*Eucalyptus globulus*	Cineol, *d*-pinene
Geranium rose oil	*Pelargonium odorata*	Geraniol, citronellol
Lavender oil	*Lavandula vera*	Linalyl acetate, linalol
Lemon oil	*Citrus limonum*	Limonene, phellandrene, citral, citronellal, linalol
Peppermint oil	*Mentha piperita*	Menthol, menthyl esters of acetic, valeric, and other acids, menthone
Rose oil	*Rosa damascena*	Geraniol, *l*-citronellol
Rosemary oil	*Rosmarinus offic.*	Pinene, camphene, cineol, camphor, borneol
Spearmint oil	*Mentha viridis*	*l*-Linalol, *l*-carvone
Thyme oil	*Thymus vulgaris*	Thymol or carvacrol
Turpentine oil	*Pinus australis, etc.*	α- and β-Pinene
Wintergreen oil	*Gaultheria procumbens*	Methyl salicylate

tilled unchanged. It will be seen from the foregoing table, which gives the constituents of these substances, that they are complex mixtures, composed for the most part of a great variety of aliphatic and aromatic compounds.

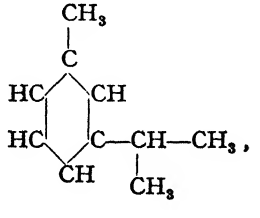
The structure of these substances has been the subject of elaborate investigation and nearly all have now been synthesised.

They may be divided into the **cyclic terpenes** and **camphors**, and a closely related group of unsaturated open-chain compounds, known as **olefinic terpenes** and **olefinic camphors.**

The Terpenes are colourless hydrocarbons, of the formula, $C_{10}H_{16}$, which are found widely distributed in the essential oils of plants. They occur in different parts of the plant, sometimes singly, sometimes two or more together, and frequently associated with allied compounds containing oxygen. More than a dozen different terpenes have been isolated, the majority being liquids, but they can all be distilled unchanged at temperatures varying from 155° to 185°. They have a high refractive index (1·46–1·47), and a low specific gravity (0·84–0·86), and, like other hydrocarbons, are insoluble in water. Many are optically active, and, as a rule, both active forms are found in nature, though in different plants. In accordance with our present knowledge of their structure, which is partly derived from their physical and chemical properties, but mainly from their synthesis, the terpenes are separated into a monocyclic and a bicyclic group. The first group may be regarded as dihydro-derivatives of *p*- and *m*-cymene, $C_{10}H_{14}$,

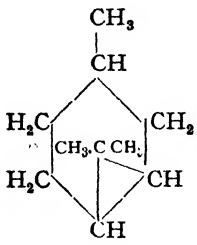


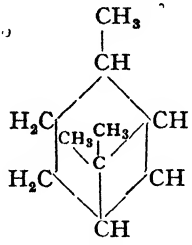
p-Cymene.

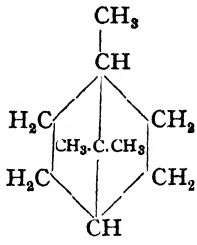


m-Cymene.

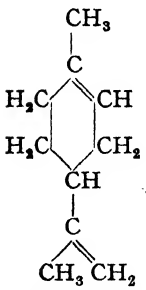
and the second as derived from the three saturated bicyclic hydrocarbons—**carane, pinane, camphane.**

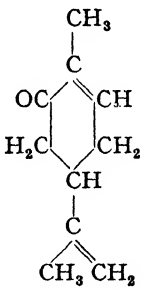

Carane.

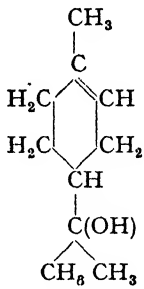

Pinane.

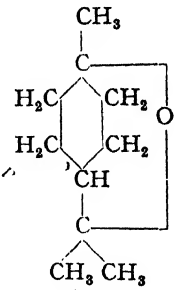

Camphane.

It is not proposed to discuss in detail either the structure or synthesis of these substances, which possess a subordinate interest in medicine; but the following formulæ are those assigned to some of the better known terpenes and the oxygen compounds related to them.


d-, *l*-Limonene (Dipentene).


Carvone.

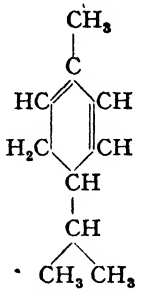

Terpineol.

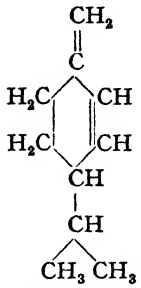

Cineol.

Limonene occurs both as the dextro- and lævo-compounds, as well as the inactive dipentene, in oil of lemons, limes, and oranges, and in many other essential oils; **carvone** is found in caraway (*Carum carvi*) and other oils, and has the characteristic smell of caraway seed; **terpineol**, which has the odour of lilac, is present in borage, cardamom, cajeput oil, etc., and **cineol** in eucalyptus, cajeput and wormseed oil (*Oleum cinae*).

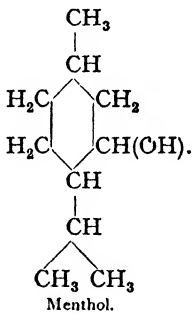
Phellandrene, another common terpene, exists in bitter- and water-fennel (*Phellandrium aquaticum*) in elemi, eucalyptus

and pine-needle oil, and is found in two modifications, α- and β-phellandrene, to which the following formulæ have been given:

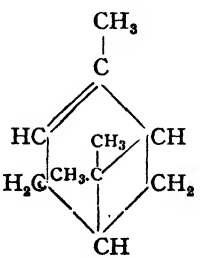

α-Phellandrene.


β-Phellandrene.

Menthol, $C_{10}H_{20}O$, which is the solid constituent present in oil of peppermint, is the hydroxy-derivative of hexahydrocymene, and has the following formula:

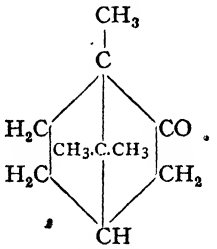

Menthol.

Pinene.—Among the bicyclic terpenes the only one of importance is pinene, having the following formula:

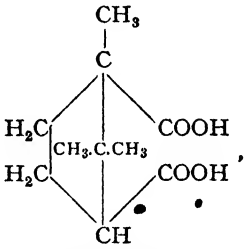

Pinene.

It is a common constituent of most essential oils and is specially abundant in the resinous exudations of different species of pinus, from which it is obtained by distillation in the form of turpentine oil, the solid residue being termed **rosin.** Two optically active modifications of pinene are known, namely, the dextro-compound, sometimes known as **australene** or American turpentine (*Pinus australis*), and the lævo-compound, or **terebenthene**, which is present in French turpentine.

Camphor, $C_{10}H_{16}O$.—Common or Japan camphor is obtained by distillation in steam from the leaves and stem of the camphor tree (*Laurus camphora*), which is cultivated in the island of Formosa and in certain districts of China. The work of numerous observers, who have studied its properties and cleavage products, has led to the formula :



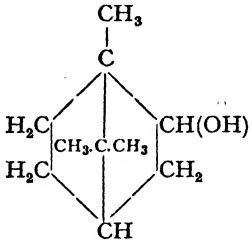
Camphor.



Camphoric acid.

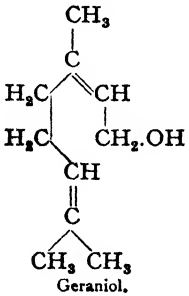
from which it will be seen that it is a ketone derivative of camphane (p. 111). On oxidation, it yields the dibasic acid, **camphoric acid.** Both substances have been synthesised. A commercial method has been introduced for the manufacture of camphor from turpentine oil. Pinene, by a series of chemical changes, not completely explained, is converted into the alcohol **isoborneol,** which yields camphor on oxidation.

Borneol or **Borneo Camphor,** $C_{10}H_{18}O$, is a natural product obtained from *Dryobanalops camphora.* It is also formed, along with the isomeric isoborneol, by the reduction of camphor, and has consequently the formula :

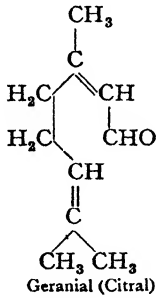


Borneol.

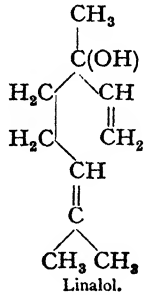
The Olefinic Terpenes and Camphors.—These substances are responsible in a great measure for the delicate aroma of the essential oils, such, for example, as the perfume of orange blossom, rose, and lavender. They exhibit among themselves a certain similarity in structure, and, at the same time, are chemically related to the terpene and camphor group. They contain ten atoms of carbon, which are disposed in such a way that six constitute a straight chain; three of them form an unsaturated isopropyl group attached to one end of the chain, and the tenth, a methyl group, is linked to the fourth carbon atom from the end of the chain. In other words, the grouping may be conceived to resemble that of a monocyclic terpene derivative in which the ring has been ruptured. The following is the probable structure of some of these compounds:



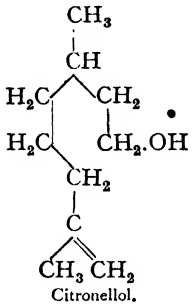
Geraniol.

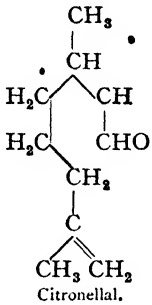


Geranial (Citral)



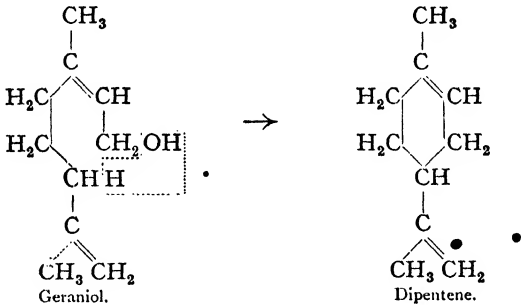
Linalol.


Citronellol.

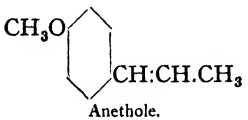

Citronellal.

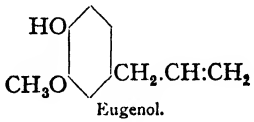
Geraniol and **Geranial** (citral) have been synthesised.

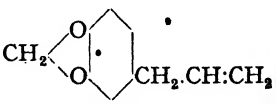
The conversion of geraniol into dipentene (p. 111) can be effected by the aid of formic acid.


Geraniol. Dipentene.

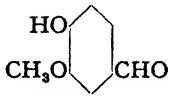
Anethole, Eugenol, Safrole, which appear among the constituents of the essential oils, are closely related aromatic compounds.


Anethole.

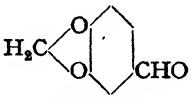

Eugenol.


Safrole.

Eugenol from oil of cloves gives **vanillin** on oxidation, whilst safrole, the chief constituent of sassafras oil, is converted into **piperonal**, which possesses the smell of heliotrope, and is known also as **heliotropin.**



Vanillin.



Piperonal
(heliotropin).

CHAPTER IX

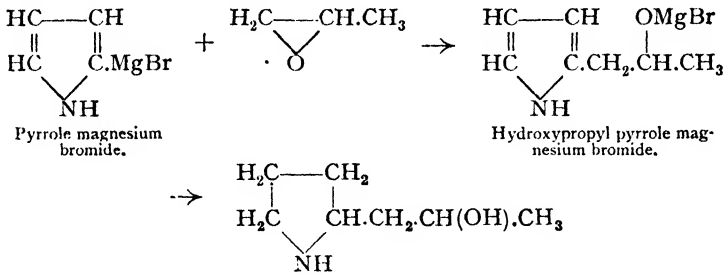
THE ALKALOIDS

Among vegetable products numerous oily and crystalline basic substances, termed **alkaloids,** have been found (see Vol. I, p. 316), which, in consequence of marked physiological properties, have been objects of special interest to the chemist and physiologist. A knowledge of their structure has led to important developments in the discovery of artificial drugs. The first of these substances to be isolated was a crystalline compound (obtained from opium by Derosne in 1803), now known as morphine. Since then the active principles of other plants have been obtained, and, indeed, scarcely a year passes without the discovery of one or more alkaloids. At the present time the number exceeds two hundred, and the field is not exhausted. The efforts of numerous investigators have shown that most of these basic substances have a pyrrole, pyridine, quinoline, or isoquinoline nucleus (Vol. I, p. 312). In view of this fact, it has been suggested that the term alkaloid should include those vegetable bases which contain a cyclic nitrogenous nucleus. The general properties of the alkaloids have already been described in Vol. I, p. 316; in the present chapter it is proposed to describe more fully the structure and synthesis of some of the more important members.

The Pyrrole Alkaloids.—The simplest of these alkaloids are **hygrine** and **stachydrine.**

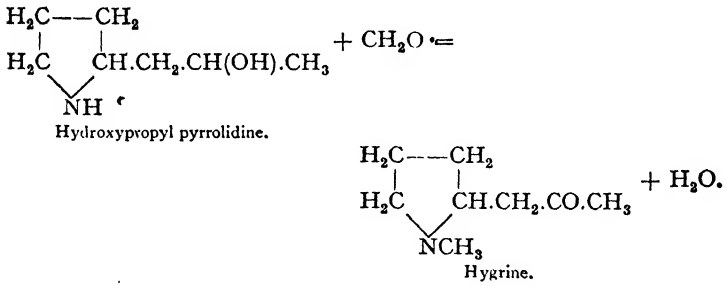
Hygrine is present in the alkaloids of coca (p. 123). It is a liquid, b.p. 193–195°, and, like the majority of alkaloids, is lævorotatory. **Stachydrine,** $C_7H_{13}O_2N + H_2O$, is a crystalline substance occurring in the root nodules of *Stachys tubifera,* and in

some other plants. Hygrine has been synthesised by Hess as follows: pyrrole magnesium bromide reacts with propylene oxide, giving hydroxypropylpyrrole:



Pyrrole magnesium bromide. Hydroxypropyl pyrrole magnesium bromide.

On reduction, four atoms of hydrogen are taken up by the ring and the corresponding pyrrolidine compound obtained. The product is methylated by the action of formaldehyde, which at the same time oxidises the carbinol group of the side-chain to the ketone and gives *r*-hygrine.



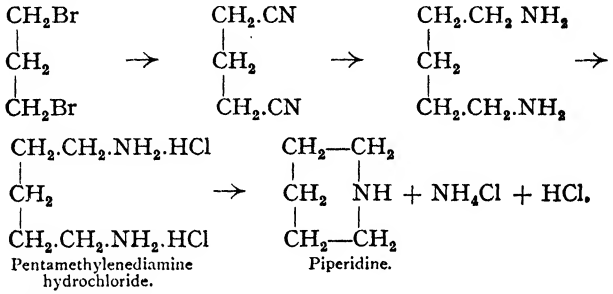
Hydroxypropyl pyrrolidine. Hygrine.

The Pyridine Alkaloids.—Among the more common of the simpler pyridine alkaloids are **piperine** and **conine**, whilst **nicotine** and **atropine** are composed of a combined pyridine and pyrrole nucleus.

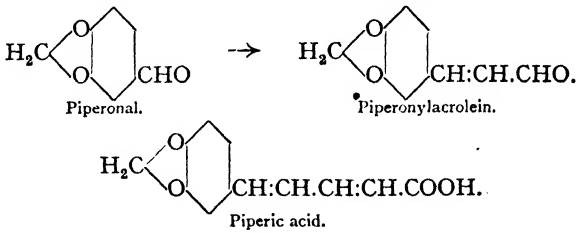
Piperine, $C_{17}H_{19}O_3N$, is contained to the extent of 7–9 per cent. in the dried seed of black pepper (*Piper nigrum*). It is a tasteless, colourless, crystalline substance which, on hydrolysis with alcoholic potash, breaks up into **piperidine** and **piperic acid**:

$$C_{17}H_{19}O_3N + H_2O = \underset{\text{Piperidine.}}{C_5H_{11}N} + \underset{\text{Piperic acid.}}{C_{12}H_{10}O_4}.$$

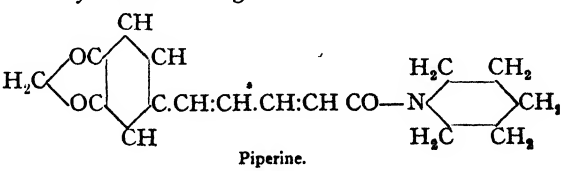
As piperic chloride reunites with piperidine to form the original alkaloid, piperine may be regarded as an amide of piperic acid. Piperidine, $C_5H_{11}N$, is a hexahydropyridine, and has been obtained from aliphatic compounds, both by the direct reduction of pyridine and by various synthetic methods. Thus, Ladenburg in 1885 prepared it by distilling the hydrochloride of pentamethylenediamine, which in turn may be obtained from trimethylene bromide. The latter is converted into the cyanide, which on reduction gives the diamine:



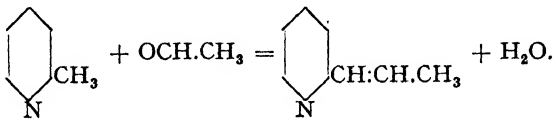
Piperic Acid has also been synthesised from piperonal by condensing it with acetaldehyde in presence of caustic soda (p. 13). The unsaturated aldehyde, piperonyl acrolein, is then converted by means of Perkin's reaction (p. 17) into piperic acid.



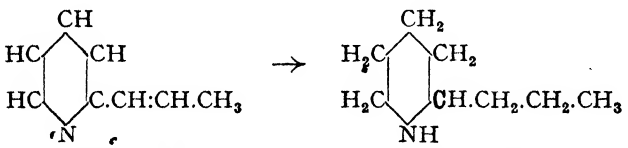
The complete structure of the alkaloid, piperine, is therefore represented by the following formula:



Conine, $C_8H_{17}N$, is the active constituent of hemlock (*Conium maculatum*) and is excessively poisonous. It occurs, associated with small quantities of other related compounds, in combination with malic acid and an aromatic acid known as caffeic acid. The largest quantity is present in the unripe seeds, from which it may be obtained by distillation with potash. It is a volatile liquid with a penetrating and unpleasant smell, and, unlike the majority of alkaloids, is dextrorotatory. It has been obtained synthetically from methyl pyridine by condensing it with acetaldehyde.



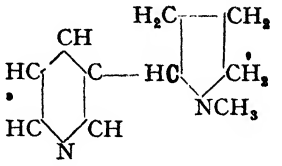
The allyl pyridine, thus obtained, is reduced in alcoholic solution with metallic sodium and is converted into propyl piperidine.



Allyl pyridine. Inactive conine

The synthetic product differs from the natural alkaloid in being inactive. The substance contains an asymmetric carbon atom (shown in thick type) and should therefore be capable of resolution into its active components. This was accomplished by Ladenburg by crystallising the bitartrate and separating the salts by fractional crystallisation (see Vol. I, p. 191).

Nicotine, $C_{10}H_{14}N_2$, has been described in Vol. I, p. 318. As there stated, it has been prepared synthetically in both active forms, the lævo-compound being identical with the natural product. The method of synthesis is a long and complex process, for details of which a special treatise must be consulted. The formula represents nicotine as a combination of a pyridine with a reduced pyrrole (pyrrolidine) nucleus.



Nicotine.

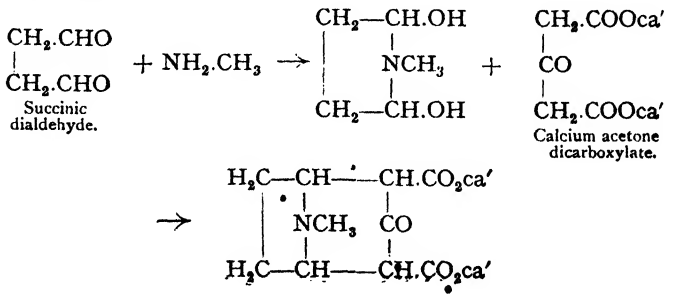
The carbon atom in thick type is the asymmetric carbon and the source of activity.

Atropine, $C_{17}H_{23}O_3N$, has also been mentioned (Vol. I, p. 318) and reference need only be made to its chemical structure. It is the ester of an alcohol, **tropine**, for, on hydrolysis with an alkali or acid, it breaks up into tropine and **tropic acid.**

$$C_{17}H_{23}O_3N + H_2O = \underset{\text{Tropine.}}{C_8H_{15}ON} + \underset{\text{Tropic acid.}}{C_9H_{10}O_3}$$

Tropine is a secondary alcohol (at the same time it has basic properties), for, on oxidation, it yields a ketone, **tropinone**, $C_8H_{13}ON$. Without discussing in detail the various steps which have led first to the knowledge of its structure and subsequently to its synthesis, mention may be made of the latest synthesis by Robinson, which possesses a peculiar interest, since it may be by some such process that tropine is formed in the plant.

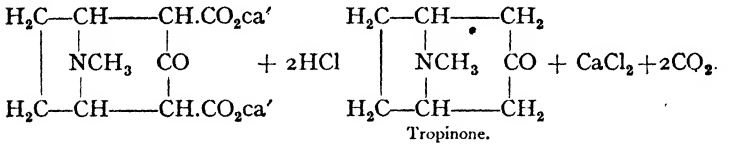
It consists in mixing succinic dialdehyde, methylamine, and the calcium salt, or ester, of acetone dicarboxylic acid. Now, succinic acid is a common plant product, whilst acetone dicarboxylic acid is readily obtained from citric acid by dehydration. There is, therefore, nothing incompatible with the idea that these substances may be present in the plant. The following condensation occurs:



Succinic dialdehyde.

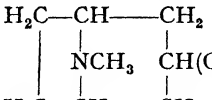
Calcium acetone dicarboxylate.

The latter, on heating with acid, gives tropinone, carbon dioxide being eliminated.



Tropinone.

On reduction, tropinone is converted into tropine.

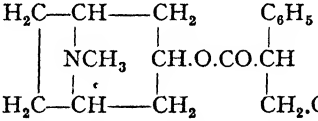


Tropine.

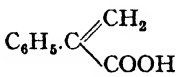
Tropic Acid, $C_9H_{10}O_3$, has also been prepared synthetically in various ways. Thus, formyl phenylacetic ester on reduction gives tropic ester.

$$\underset{\text{Formyl phenylacetic ester.}}{C_6H_5.CH(CHO).COOC_2H_5} \rightarrow \underset{\text{Tropic ester.}}{C_6H_5.CH(CH_2OH).COOC_2H_5}.$$

Tropic acid contains asymmetric carbon and exists in dextro- and lævo-forms. In atropine, tropine is combined with the inactive acid, but in hyoscyamine it is united with the lævo-compound. The following then is the formula for atropine:



Apoatropine is tropine combined with atropic acid, a dehydration product of tropic acid.



Atropic acid.

whilst **tropa-cocaine**, found in cocaine (see below), is the benzoic ester of the isomeric ψ-tropine.

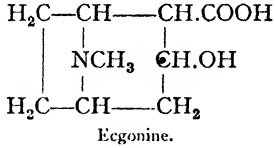
It should be pointed out that tropine has been combined with a variety of aromatic acids, many of which possess a mydriatic action on the pupil of the eye. They are termed **tropeïnes.**

The tropeine of mandelic acid is used in ophthalmic practice under the name of **homatropine.** Also a variety of synthetic drugs of a certain structure have been found to possess a similar action (see p. 134).

Cocaine, $C_{17}H_{21}O_4N$.—The leaves of *Erythroxylon coca* contain a series of alkaloids, among which *l*-**cocaine,** *d*-**cocaine, tropacocaine, cinnamyl-cocaine** α- and β-**truxilline** and **hygrine** have been identified as distinct constituents. *l*-Cocaine, the most abundant and, physiologically, the most valuable constituent, was isolated by Niemann in 1860; but it is only in recent years that its important physiological properties as a local anæsthetic have come into prominence. Like atropine, it is a crystalline, tertiary base, and also like atropine it breaks up, on hydrolysis, into a new tertiary base, *l*-**ecgonine,** benzoic acid, and methyl alcohol.

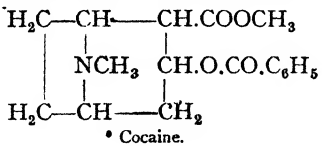
$$C_{17}H_{21}O_4N + 2H_2O = C_9H_{15}O_3N + C_6H_5.COOH + CH_3.OH.$$

By a reversal of the process, *l*-cocaine has been reconstructed from *l*-ecgonine. The structure of cocaine therefore depends upon that of ecgonine. Its synthesis has been effected, using tropinone as the starting point. Tropinone sodium, when suspended in ether and heated with carbon dioxide, forms sodium tropinone carboxylate, and the latter, on reduction with sodium amalgam, is converted into inactive ecgonine.



Ecgonine.

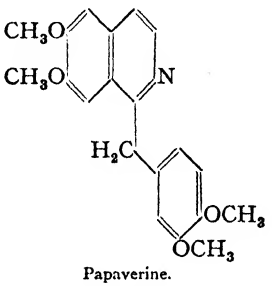
At the same time, an isomeric ψ-**tropine** is formed. Cocaine will therefore be represented by the following formula:



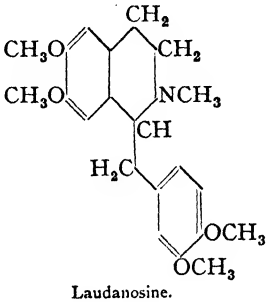
Cocaine.

The Isoquinoline Alkaloids.—Among the numerous alkaloids found in opium (Vol. I, p. 320) several have been

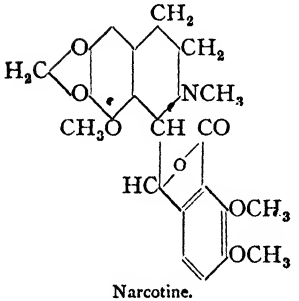
shown to contain an isoquinoline nucleus, namely, **papaverine, laudanosine, narcotine** and **narceïne,** whilst **hydrastine** and **berberine** occur in the roots of the golden seal (*Hydrastis canadensis*) and elsewhere. It is not proposed to discuss in detail the structure of these compounds. The majority of them have now been synthesised and their structure is fully ascertained. It must suffice, therefore, to give their formulæ, from which their close relationship will be seen.



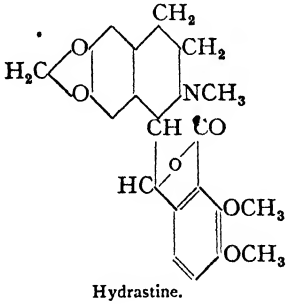
Papaverine.



Laudanosine.



Narcotine.

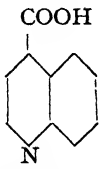


Hydrastine.

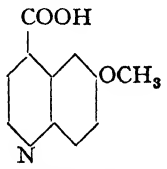
The Quinoline Alkaloids.—These may be subdivided into the cinchona and strychnos alkaloids.

The Cinchona Alkaloids.—In spite of a large mass of research which has been concentrated on the active constituents of cinchona bark, the structure of neither quinine nor cinchonine is yet definitely proved, nor has either substance been obtained synthetically. It is known, for example, that, on distillation with caustic alkalis, quinoline is obtained, and by oxidation the two

alkaloids break up into quinoline derivatives, cinchonine giving cinchoninic acid and quinine the closely related quininic acid which have been shown to have the following structure;

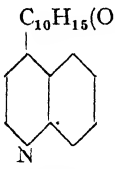


Cinchoninic acid.

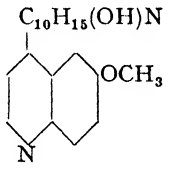


Quininic acid.

As the two acids stand in the same relation as the alkaloids, it would appear that the other part of the molecule is identical in the two substances, which may therefore be represented by the following general formula:



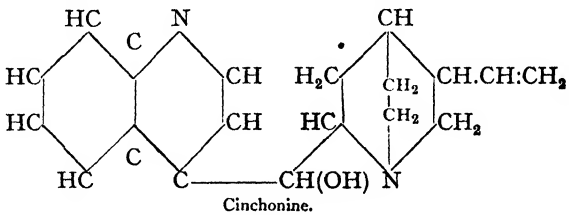
Cinchonine.



Quinine.

The investigation of the second half of the molecule has presented unforeseen difficulties, and its constitution is not yet established.

There are, however, good grounds for assuming that the formula of the cinchonine molecule is represented as follows:



Cinchonine.

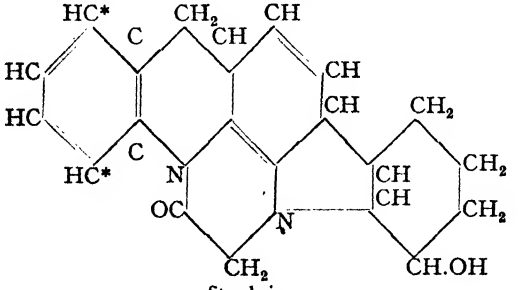
whilst that of quinine will be the methoxy-derivative.

EXPT. 44.—**Quinine Sulphate from Cinchona Bark.**—Slake 20 grams of quicklime and mix into a thin cream with 200 c.c. of water. Pour the liquid into a basin containing 100 grams of cinchona bark previously ground in a coffee mill, and stir up the mass. Dry the mixture thoroughly on the water-bath, taking

care to powder the lumps that ball together. When cold, place the powder in a flask, pour over it 200 c.c. of chloroform, and let the mixture stand overnight. Filter through a porcelain funnel and wash with a further 200 c.c. of chloroform. The chloroform solution, which has now a faint yellow colour, is shaken well with 50 c.c. and again with 25 c.c. of dilute sulphuric acid, and then with water until the aqueous solution loses its blue fluorescence. The combined acid and aqueous extracts are carefully neutralised with ammonia, and the liquid concentrated on the water-bath until crystals of quinine sulphate begin to form on the surface. The liquid is allowed to cool and filtered. A further quantity of crystals may be obtained from the mother liquor by evaporation, but the product is not so pure. The quinine sulphate is purified by re-crystallisation from water. The yield is 1 to 2 grams, according to the quality of the bark.

Reactions.—Use a solution of the hydrochloride, prepared by adding a few drops of hydrochloric acid to the sulphate mixed with water. Add to a little of the solution a few drops of iodine solution (iodine dissolved in potassium iodide). A brown amorphous precipitate is formed. This is a general reaction for alkaloids. Add chlorine water and then ammonia in excess. An emerald green colouration is produced. Add sodium carbonate solution and then shake with ether. The free base is precipitated and dissolves in the ether. Decant the ether on to a watch-glass and let it evaporate. Crystals of the base remain. Dissolve in a few drops of acetic acid and add water. A blue fluorescent liquid is obtained.

The Strychnos Alkaloids are apparently still more complex in structure; their elucidation is still more remote. Sufficient information has, however, been collected to lead to the following provisional formula for **strychnine**:

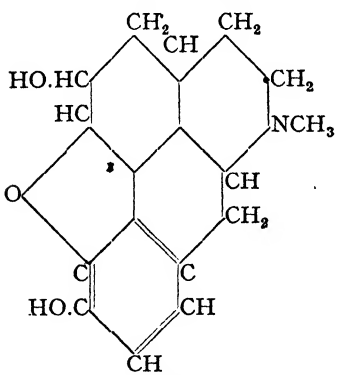


Strychnine.

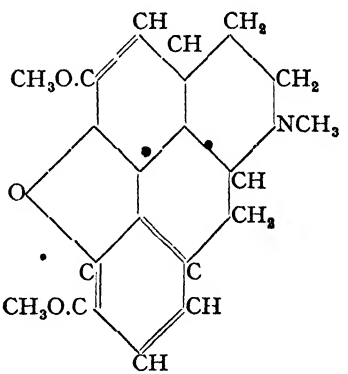
Brucine is the dimethoxy-derivative, the two methoxyl groups replacing the hydrogen of the two carbons with the asterisk.

The Morphine Alkaloids.—This group includes at least four important alkaloids found in opium, namely, **morphine, codeïne, ψ-morphine** and **thebaïne.** They are distinguished from the more numerous class of opium alkaloids (to which papaverine and narcotine belong) by their poisonous character. In spite of the enormous mass of material which has resulted from the study of these alkaloids, we are still ignorant of their structure. **Morphine** and **codeïne,** which are represented by the formulæ $C_{17}H_{19}O_3N$ and $C_{18}H_{21}O_3N$ differ clearly by one methyl group, whilst ψ-morphine, $C_{17}H_{18}O_3N$, is an oxidation product of morphine, and can be prepared from the latter by the action of weak oxidising agents. **Thebaïne,** $C_{19}H_{21}O_3N$, is the dimethoxy-derivative of morphine less two hydrogen atoms.

The following provisional formulæ have been assigned to morphine and thebaïne:



Morphine.



Thebaïne.

CHAPTER X

SYNTHETIC DRUGS

THE study of pharmacology has relation to the physiological effects of certain chemical substances, the majority of which find their place among organic compounds.

The preparation of synthetic drugs had its origin first in the isolation of the active principles of certain medicinal plants, and secondly in the study of their structure.

Thus, the isolation of cocaine from coca leaves not only established the nature of the active principle, but the knowledge of its structure led to the synthesis of a variety of even more valuable substances having similar properties.

In this way it was shown that a certain relation exists between structure and physiological properties. The relation is, however, by no means well defined, owing partly to the complexity of the organism with which they are brought into contact, and partly to the physical as well as the chemical nature of the substances themselves. Solubility in water, or in the animal fluids, and consequent rate of absorption, as well as volatility, are factors portance. Moreover, the drug may, in addition to its specific on, set up some secondary effect by acting on certain tissues ssage through the system.

dose may produce one result, whilst a larger dose by its ion may bring about a different and in some cases an site effect.

, it is possible to guard against this indirect action. e gastric juice of the stomach, containing a minute chloric acid, cannot hydrolyse esters which the of the intestine is capable of effecting. In

this way salicylic acid, which has an irritant action on the stomach, may be rendered innocuous by conversion into its phenyl ester (salol) which is only hydrolysed on reaching the intestine, where the free acid is then able to perform its specific function. It is an interesting fact that among optically active compounds the two active forms may exert very different effects. This has been observed in the case of *d*- and *l*-nicotine, the lævo-compound being much the more toxic; in the case of the two active hyoscyamines, of which the lævo-compound has one hundred times the mydriatic effect of the dextro-compound, and in that of the two adrenalines, the lævo-compound again being physiologically the more potent.

Such knowledge can only be obtained by experiment, and it is by a careful comparison of the action of a series of similarly constituted compounds on the living organism that these highly complex problems can be attacked.

In spite of the difficulties, which have naturally attended this branch of investigation, progress has been continuous, and the number of new and valuable synthetic drugs has steadily increased.[1]

An attempt has been made by Ehrlich and others to discover a theory underlying their action. According to Ehrlich, certain groups in the molecule possess the power of attaching or anchoring themselves to the cells upon which they act, after the manner of certain dyestuffs which attach themselves to particular fibres. These anchoring groups enable the drug to exert its specific action.

If this anchoring group is removed or modified, a second anchoring group may come into action and attach itself to some other tissue with entirely different results. An acid group (CO_2H or SO_3H) may suspend the activity of an anchoring group until esterification renders it inactive. Benzoyl ecgonine only exerts its action as a local anæsthetic when the adjoining carboxyl group is esterified (p. 133). Whatever may be the cause, it is remarkable how slight a change in structure will completely alter the physiological effect; whilst, on the other hand, substances of a totally different constitution may possess almost identical physiological properties.

[1] Pyman, *Trans. Chem. Soc.*, 1917, **111**, 1103.

The principal classes into which drugs may be divided are **narcotics, antipyretics, antiseptics, mydriatics,** those which increase the blood pressure or **sympatho-mimetics;** and **protozoacides** which destroy blood parasites.

Narcotics.

Narcotics, which include anæsthetics and hypnotics, act upon the nerve-centres, inducing sleep, or, in larger doses, insensibility. The aliphatic hydrocarbons, and to a greater degree the unsaturated hydrocarbons of the ethylene, acetylene, and benzene series, have a narcotic action which increases with their volatility and solubility in water, so that the higher, less volatile, and less soluble members are inert.

Many of the aliphatic alcohols have a narcotic action; but whilst methyl alcohol is inactive, ethyl alcohol in large doses produces sleep. The narcotic effect appears to increase with the number of branches in the carbon chain, so that tertiary alcohols are more active than secondary or primary alcohols. At the same time the nature of the radicals present is an important factor, and in this respect ethyl is more potent than methyl. Whereas tertiary amyl alcohol, $(CH_3)_2C_2H_5.C.OH$, with one ethyl group is active, tertiary butyl alcohol, $(CH_3)_3C.OH$, with three methyl groups is not. The same activity of the ethyl group is observed in other hypnotics such as ether, sulphonal, and certain ureides.

The narcotic power of the alcohols is preserved to a certain degree in the aldehydes. Paraldehyde (Vol. I, p. 51) is a useful hypnotic, being less irritant than acetaldehyde.

Many of the more volatile halogen derivatives of the aliphatic hydrocarbons have an anæsthetic action, due to the presence of the halogen. The activity of the chlorine derivatives of methane increases with each additional chlorine atom from methyl chloride to carbon tetrachloride.

Chloroform has already been described (Vol. I, p. 102) as a valuable anæsthetic. Its admixture with ethyl chloride and bromide is known as **somnoform.** Though carbon tetrachloride has a stronger action, it is associated with increased toxicity.

Chloral hydrate, another important hypnotic, has been described in Vol. I, p. 113. Various derivatives of chloral are also employed, such, for example, as chloral formamide or **chloralamide** and **dormiol**, a condensation product with tertiary amyl alcohol:

$CCl_3.CH(OH).NH.CHO$ — Chloralamide.

$CCl_3.CH(OH).O.C_5H_{11}$ — Dormiol.

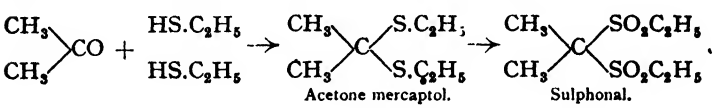
Other hypnotics are **butyl chloral**, obtained by passing chlorine into acetaldehyde, and **chloretone**, a condensation product of acetone and chloroform, prepared by the action of potash on a mixture of these two substances:

$CH_3.CHCl.CCl_2.CHO$ — Butyl chloral.

$CCl_3.C(OH)(CH_3)_2$ — Chloretone.

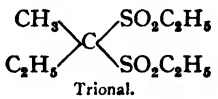
The latter is often used as a specific against sea-sickness. In addition to the above are two further classes of hypnotics, namely, the sulphones and the amides.

Sulphonal is prepared by condensing acetone with ethyl mercaptan, $C_2H_5.SH$, the sulphur analogue of ethyl alcohol, and then oxidising the product with permanganate:

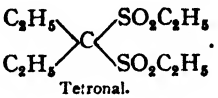


Acetone mercaptol. Sulphonal.

Trional and **tetronal**, which possess a similar but somewhat greater activity, are made respectively from ethyl, methyl, and diethyl ketones:



Trional.

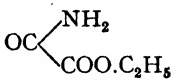


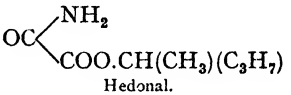
Tetronal.

The activity of these substances seems to depend on the presence of an ethyl radical either in the methylene or sulphone group, for neither $CH_2(SO_2C_2H_5)_2$ nor $CH_3.CH(SO_2CH_3)_2$ have any

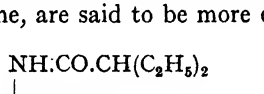
hypnotic action, and though the compound $C_2H_5.CH(SO_2CH_3)_2$ is distinctly hypnotic, its effect is slight.

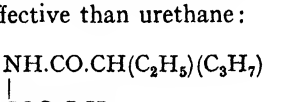
Of the second class of hypnotics the most important are **urethane** and its derivatives, **adalin** and **veronal**. **Urethane** and **hedonal**, the secondary amyl (methyl propyl carbinol) ester, have been referred to (Vol. I, p. 221):


Urethane.


Hedonal.

Other derivatives, such as the diethylacetyl- and ethyl propyl-urethane, are said to be more effective than urethane:





Adalin is diethyl bromacetyl urea,

$$(C_2H_5)_2CBr.CO.NH.CO.NH_2,$$

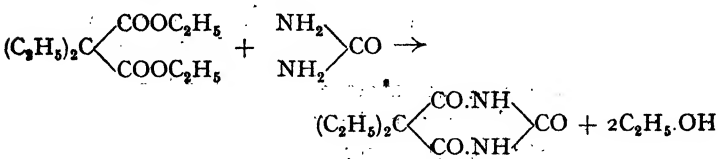
and is prepared from malonic ester, which is first converted into the diethyl derivative (see p. 7), then into diethylacetic acid which is brominated, and converted into the urea derivative:

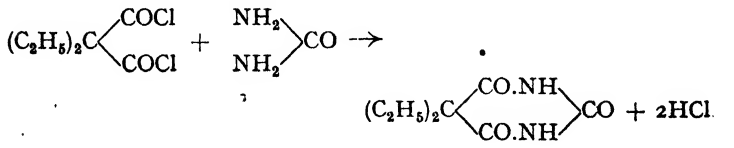
$$CH_2(CO_2.C_2H_5)_2 \rightarrow (C_2H_5)_2.(CO_2H)_2 \rightarrow (C_2H_5)_2CH.CO_2H \rightarrow (C_2H_5)_2CBr.CO_2H.$$

α-Bromodiethyl acetic acid.

it is less toxic than veronal (see below).

Veronal, or diethylmalonyl urea, one of the most valuable hypnotics, is prepared by combining diethylmalonic ester with urea in presence of sodium ethoxide, or by the action of urea on diethyl malonyl chloride:





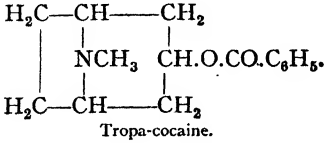
Whilst the monoethyl and dimethyl derivatives are quite inert, the dipropyl derivative, or **proponal**, has an increased action, but a more prolonged after-effect.

It is an interesting fact that the replacement of the hydrogen of the NH group by a radical produces a highly toxic substance. Local anæsthesis may be produced by freezing the surface of the skin by spraying it with some highly volatile liquid such as low boiling petroleum ether, methyl and ethyl chlorides, etc., but there are a number of chemical substances such as cocaine and related compounds which cause local insensibility and are employed for small operations.

Cocaine has already been referred to (p. 123) as being obtained from the leaves of *Erythroxylon coca*.

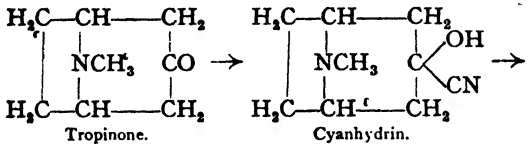
As neither benzoyl ecgonine nor ecgonine methyl ester possesses any anæsthetic action, the property seems to reside not merely in the benzoyl radical (which may be replaced by other aromatic acyl radicals) but in its association with an ester group (not necessarily restricted to the methyl ester, see p. 129).

Tropa-cocaine, which is also a constituent of the coca leaf, is an even more powerful anæsthetic and less toxic than cocaine, though it contains no ester group.

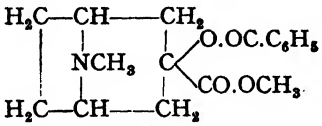


Tropa-cocaine.

On the other hand, α-cocaine, which is obtained from tropinone (see p. 122) by the action of hydrogen cyanide and subsequent conversion of the hydroxy-acid into the methyl benzoyl ester is devoid of anæsthetic properties.

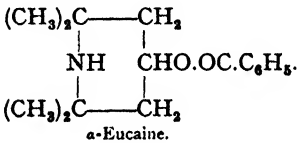


Tropinone. Cyanhydrin.

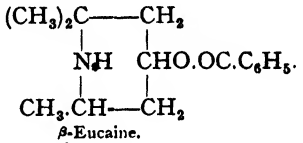


α-Cocaine.

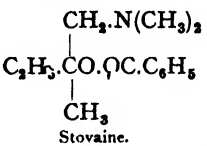
A knowledge of the structure of cocaine has led to many successful attempts to produce synthetic drugs of similar constitution. Among these may be mentioned **α-** and **β-eucaine, stovaine, alypine, novocaine, holocaine, orthoform, nirvanine,** etc., having the following formula :



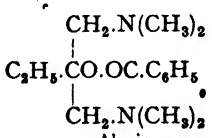
α-Eucaine.



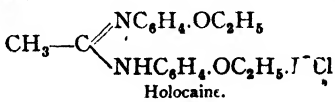
β-Eucaine.



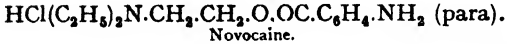
Stovaine.



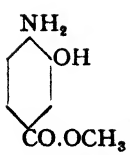
Alypine.



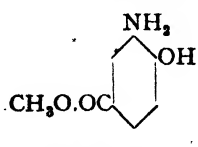
Holocaine.



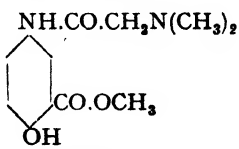
Novocaine.



Orthoform.



New orthoform.

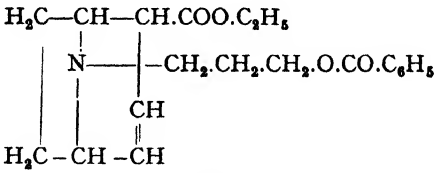


Nirvanine.

It will be seen from the above that the only essential part of the structure producing local anæsthesia is the ester of an amino-

acid of the general formula NRR.CRR.COOR, in which the acyl group contains an aromatic nucleus.

J. v. Braun has made the interesting observation that by replacing the methyl group of the central nitrogen atom in anhydroecgonine by a propyl benzoate chain such as that in tropacocaine, the anæsthetic properties are not only increased, but the intense toxicity of cocaine is removed.

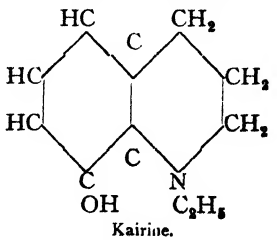


Eccaine.

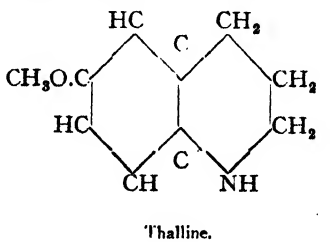
The substance has been named **eccaine.**

Antipyretics.

One of the earliest attempts at the synthesis of an antipyretic compound followed the discovery that quinine contained a methoxyquinoline nucleus. From the fact that tetrahydroquinoline is physiologically more active than quinoline, O. Fischer and Filehne were led to prepare kairine and thalline by Skraup's reaction (Vol. I, p. 314), the former from *o*-aminophenol and the latter from *p*-methoxyaniline. The products, on reduction, yield the corresponding tetrahydro-derivatives. In the case of kairine the imino-group of the nucleus was further ethylated.



Kairine.

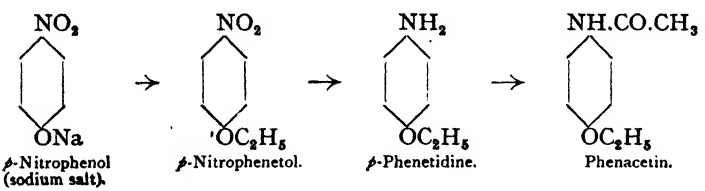


Thalline.

They proved, however, unserviceable, owing to their toxic properties, and were soon replaced by antipyrine (p. 18), or

phenyl dimethylpyrazolone, which is more active than quinine though of little use against malaria, and **pyramidone**, the dimethylamino-derivative which is more powerful than antipyrine.

Aniline (Vol. I, p. 262) and **acetanilide** (Vol. I, p. 268) have also antipyretic and anti-neuralgic properties, but whereas the former is too toxic to be used, the latter, under the name of **antifebrin**, often forms a constituent of headache powders on account of its cheapness. **Exalgine**, its methyl derivative, $C_6H_5N(CH_3).CO.CH_3$, is also occasionally used, but, like acetanilide, is not a safe remedy. Much more effective are the derivatives of *p*-aminophenol, $OH.C_6H_4.NH_2$. Attention was directed to this substance by the fact that it is formed from aniline in the body, and like other substances which are rendered less toxic by their passage through the system (p. 146), aminophenol, whilst retaining the antipyretic properties of aniline, was distinctly less poisonous. Nevertheless, it proved unsatisfactory. The acetylation of the amino-group lowers the toxicity, which is further reduced in the methoxy- and ethoxy-derivatives, whilst the antipyretic and anti-neuralgic effects are increased. These acetyl derivatives are known as **methacetin** and **phenacetin**, the latter having maintained its pre-eminence since its first introduction. It is obtained from *p*-nitrophenol by the following series of changes: the sodium salt is heated with ethyl bromide and the nitro-group then reduced; finally, *p*-ethoxyaniline (phenetidine) is acetylated.

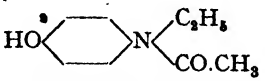


p-Nitrophenol (sodium salt). *p*-Nitrophenetol. *p*-Phenetidine. Phenacetin.

It can also be prepared by the ethylation of *p*-acetylamino-phenol.

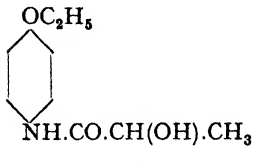
By substituting radicals of higher molecular weight in place of the ethoxyl group, the antipyretic action is weakened. Again,

if the alkyl radical of the hydroxyl group is transferred to the amino-group, the product loses its activity:

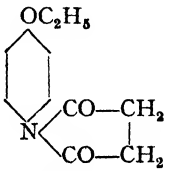


p-Ethylacetylaminophenol.

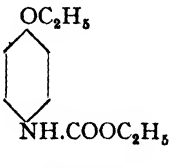
Other acyl groups may, however, replace the acetyl group, such as lactyl, succinyl, carbethoxyl, glycyl, etc., yielding a series of compounds with more or less pronounced antipyretic properties:



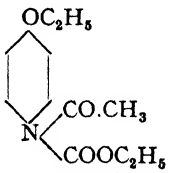
Lactophenin.



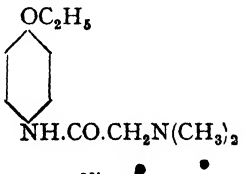
Pyrantin.



Thermodin.



Neurodin.



Nirvanin.

Antiseptics.

The term antiseptic implies the power of inhibiting the growth of germs without necessarily destroying them. A substance possessing this property is boric acid. But the word is now applied indifferently to substances which both inhibit and kill, and is synonymous with disinfectant and germicide.

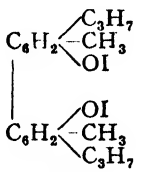
Antiseptics may be divided into four classes, the halogen compounds, the antiseptic action of which depends on the presence of free or combined halogen, the phenol antiseptics, the antiseptic dye-stuffs, and a few which fall into none of these groups.

Halogen Antiseptics.—The halogens have long been recognised as possessing a powerful antiseptic action; iodine itself,

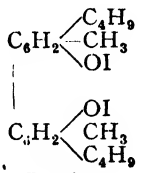
and such substances as the hypochlorites, which readily attack protein matter, are among the most active germicides. We are, however, concerned here more especially with organic compounds.

Iodoform is extensively used in surgery for dressing wounds (Vol. I, p. 103); but its unpleasant and penetrating odour has led to the introduction of various substitutes. **Iodoformin,** for example, is the compound with hexamine (Vol. I, p. 73), and is odourless, so also is **iodol** or tetraiodopyrrole (p. 118), both of which act by liberation of iodine.

Tri-iodo *m*-cresol (**losophan**), and the iodoxy-compounds known as **aristol** (thymol di-iodide) and **europhen** have strong antiseptic properties.



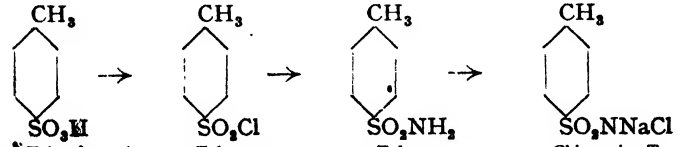
Aristol.



Europhen.

Sozoiodol, $C_6H_2I_2(OH)SO_3K$, **isoform** (iodoxyanisol), $C_6H_4.OCH_3.IO_3$, and **iodoguaiacol** may also be included.

The chloramines form another group of organic compounds containing chlorine. They were first prepared by Chattaway and subsequently introduced by Dakin and his collaborators as antiseptics for the treatment of wounds. **Chloramine-T** is prepared from *p*-toluene sulphonic acid (Vol. I, p. 25) by conversion into the chloride and amide. The latter combines with sodium hypochlorite to give the sodium salt of the sulphonchloramide, which is crystalline and readily soluble in water:



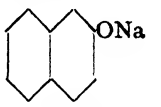
p-Toluene sulphonic acid. *p*-Toluene sulphonic chloride. *p*-Toluene sulphonamide. Chloramine-T.

Another derivative, **dichloramine-T**, is prepared from the sulphonamide by the action of sodium hypochlorite, and, though decomposed by water, can be used in a solution of chlorinated paraffin or eucalyptol. **Halazone** is the dichloramine of sulphonbenzoic acid,

Halazone.

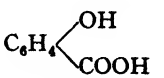
and is used for sterilising drinking water.

Phenol Antiseptics.—The replacement of hydrogen in aromatic hydrocarbons by hydroxyl produces substances having in a greater or less degree antiseptic properties. The property is intensified by increasing the number of hydroxyl groups, and also by introducing halogen atoms, but is accompanied by increased toxicity. Methyl groups, whilst increasing the antiseptic effect, renders them less poisonous. On the other hand, their solubility is diminished. Such substances as cresol, $C_6H_4(CH_3)OH$, have, therefore, to be used as soap emulsions (**lysol**) or dissolved in a suitable solvent. β-Naphthol (Vol. I, p. 305) is also used as the sodium salt under the name of **microcidin.**

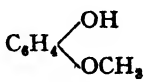


Microcidin.

Whilst these substances are useless for internal application, the entrance of a carboxyl or methoxyl group into the ortho-position (the meta and para positions are ineffective), though it diminishes the antiseptic action, lowers the toxicity, and both salicylic acid or its sodium salt (Vol. I, p. 298) and guaiacol are useful internal antiseptics:



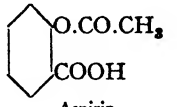
Salicylic acid.



Guaiacol.

Salicylic acid, it may be added, acts also as an antipyretic and anti-rheumatic. Salicylic acid, however, often disturbs the digestive system, and has consequently been replaced by

aspirin, the acetyl derivative, which is only hydrolysed on reaching the intestine:



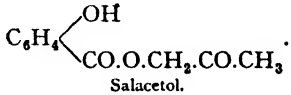
Aspirin.

Many other acyl derivatives of salicylic acid have been prepared having similar properties, but greater solubility. Nevertheless, aspirin maintains its popularity.

Another series of antiseptics derived from salicylic acid are the **salols**, in which the carboxyl hydrogen is replaced by phenolic and other radicals.

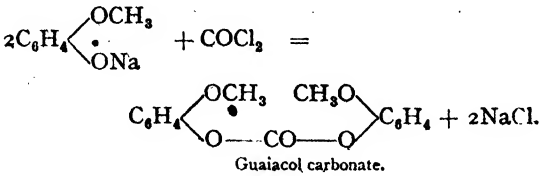
Salol is phenyl salicylate (Vol. I, p. 299), and is prepared by the action of phenol on salicylic acid in presence of phosphorus- or carbonyl-chloride; **betol** is a similar derivative of β-naphthol. They are gradually hydrolysed in the intestine into the phenol and salicylic acid, both of which act as antiseptics and are slowly absorbed by the system.

Among the "partial salols," as they are termed, are esters of physiologically active acids, and inactive alcohols or inactive acids and active phenols. Belonging to the former class are methyl salicylate (**wintergreen oil**), the monoglyceric ester (**glycosal**), the glycol ester (**spirosal**), and the acetone ester (**salacetol**), obtained by the action of chloracetone on sodium salicylate.



Salacetol.

Belonging to the latter class are **guaiacol** and its esters. Guaiacol is the monomethyl ether of catechol (Vol. I, p. 285) and is much less toxic than the parent phenol; but, like salicylic acid, it has an irritant action on the stomach, and is therefore used in the form of the carbonate which, like aspirin and salol, undergoes hydrolysis in the intestine, giving guaiacol and carbon dioxide. The carbonate (**duotal**) is prepared by the action of carbonyl chloride on sodium guaiacol.

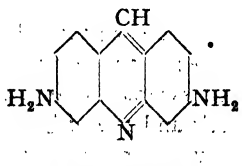


Guaiacol carbonate.

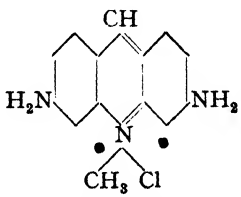
A variety of guaiacol esters are also used, such as the benzoate (**benzosol**), the acetate (**eucol**), and the cinnamate (**styracol**).

Antiseptic Dyestuffs.—Certain dyestuffs have been shown to possess not only a destructive action on blood parasites of protozoal origin, such, for instance, as cause sleeping sickness and malaria, but to act as true antiseptics in their power of destroying micro-organisms.

Among those of the latter class, which have come into prominence in recent years, are malachite and brilliant green, methylene-blue and the yellow acridine dyestuffs, namely diaminoacridine sulphate (**proflavine**) and its methochloride (**acriflavine**).



Diamino-acridine.



Acriflavine.

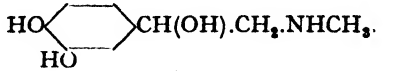
Unclassified Antiseptics.—Of the other antiseptics, not included in the above categories, are formaldehyde and its polymeric derivative, **paraform** (Vol. I, p. 71), as well as compounds of formaldehyde with gelatine (**glutol**), with dextrin (**dextroform**), and lactose (**formamint**). **Hexamine** or **urotropine**, which is the crystalline compound formed by the combination of formaldehyde with ammonia (Vol. I, p. 73), is employed as a urinary antiseptic. Tannic and gallic acids are antiseptics. The unpleasant taste of tannic acid is masked in the acetyl derivative, **tannigen**, and by its union with formaldehyde, **tannoform**. Bismuth salts of tannic acid are also used under the names of **dermatol** and **airol**.

Testing of Antiseptics.—The toxic effect of antiseptics is determined by giving weighed doses to small animals, and their irritant action by exposing small surfaces of the skin to the substance and recording the effect. The bactericidal action is observed by introducing into a series of test-tubes a solution of the antiseptic in progressively decreasing concentration, and adding to each, a drop of a twenty-four hour old broth culture of a specific organism (*Staphylococcus* or *B. coli*). The tubes are plugged, shaken up, and left at the ordinary temperature for two hours. A loop full of the contents of each tube is then added to another series of tubes containing the same volume of sterilised broth and incubated for twenty-four hours at 37°. If no growth appears, sterilisation is complete. If there is a growth, the number of microbes are estimated by transferring a portion to Petri dishes at certain intervals, and counting the colonies in the usual way.

Sympatho-mimetics (Adrenaline bases).

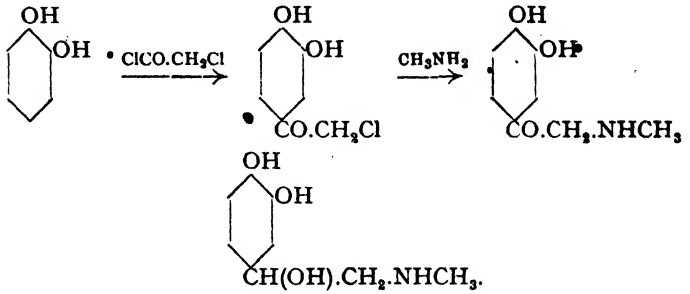
Adrenaline is the active principle of the suprarenal gland, which resembles in its action the effect of exciting the sympathetic nerves, and has therefore been termed sympatho-mimetic. It produces constriction of the blood-vessels and rise of blood pressure (pressor action) when administered subcutaneously or locally, and is used for stopping bleeding. This is its chief therapeutical application.

Its structure has been carefully studied, and it is now known to have the following formula:



Adrenaline.

It has been prepared synthetically in various ways, the earliest being that of Dakin and Stolz, who obtained it by combining catechol with chloracetyl chloride, acting upon the latter with methylamine, and then reducing the resulting ketone (**adrenalone**) with the aluminium–mercury couple or by electrolysis.

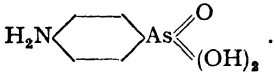


This discovery has led to a very complete study of the activity of substances of similar structure by observing the pressor effect. Barger and Dale have shown that the properties of adrenaline are shared to a greater or less extent by a number of both aliphatic and aromatic amines, but the most active of the simpler bases is phenylethylamine, $C_6H_5.CH_2.CH_2.NH_2$, the parent substance of adrenaline. The presence of two hydroxyl groups in the 3:4 position of the nucleus increases the effect, which is further intensified by a hydroxyl group in the side-chain. In short, the natural product seems to be the best adapted for this special function. Among the substances examined which have a pronounced pressor effect are several which are derived from naturally occurring amino-acids by loss of carbon dioxide; such are isoamylamine (from leucine), *p*-hydroxyphenylethylamine (from tyrosine), indole ethylamine (from tryptophane), and aminoethyl glyoxaline (from histidine) (p. 72), but they are all inferior in activity to adrenaline, the order being as follows: *p*-hydroxyphenylethylamine has about one-twentieth the effect of adrenaline, then phenylethylamine, isoamylamine and isobutylamine.

Organo-arsenic Compounds.

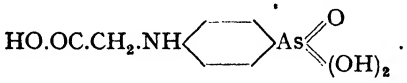
Some diseases, such as sleeping sickness and syphilis, have been traced to the presence in the blood of protozoal parasites. These parasites have been found to be very sensitive to certain organic derivatives of arsenic. The first of these was discovered by Béchamp in 1863 by heating aniline with arsenic acid, and

regarded by him as the anilide of arsenic acid. It was subsequently found, when injected into patients suffering from sleeping sickness, to have a remarkable curative effect, and was named **atoxyl** from its comparatively non-poisonous character. Bertheim and Ehrlich, who later examined the structure of the substance, found that it underwent the usual reactions of a primary amino compound, such as aniline, giving the ordinary diazo-reactions, etc. In this way it was recognised that the compound was not an anilide but *p*-amino-arsinic acid.

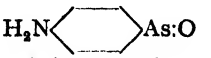


Atoxyl (aminoarsinic acid).

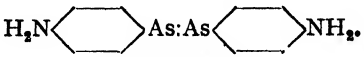
Its mono-sodium salt, which was the one generally used, is soluble in water. It rapidly destroys the trypanosomes of sleeping sickness, but is liable to produce blindness. From the fact that the introduction of an acetyl group into *p*-aminophenol diminishes the toxic effect of this substance (p. 136), the acetyl derivative of atoxyl or **arsacetin** was prepared with the same object and gave the desired result. The glycine ester is even more satisfactory,



Although atoxyl is able to destroy trypanosomes in the blood of the patient, it has little effect on the parasite outside the animal organism. Ehrlich therefore concluded that atoxyl undergoes some chemical change in the body, which confers upon it its special attribute. As this change is frequently one of reduction, atoxyl was submitted to the action of reducing agents, with the result that two compounds, namely, aminoarsenic oxide and aminoarsenobenzene, were obtained:



Aminoarsenic oxide.



Aminoarsenobenzene.

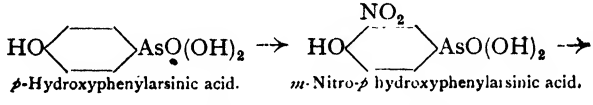
The first is highly toxic, but is rendered much less so by conversion into the hydroxy-derivative, whilst its trypanocidal properties are enhanced, a strength of one in ten million being

capable of destroying the trypanosomes *in vitro*, whereas a strength of 5 per cent. of atoxyl is without action. Still less toxic is the arsenobenzene derivative, whilst giving a higher trypanocidal effect.

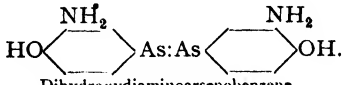
It seems probable, therefore, that atoxyl is reduced in the body, and, according to Ehrlich, its action is due to the presence of tervalent arsenic which, as an unsaturated atom, can attach itself to certain cells of the parasite.

The success of these experiments has led to the preparation of a great variety of arsenobenzene derivatives. Of these, the most valuable are **salvarsan** and **neosalvarsan**, which are used in the treatment of syphilis.

Salvarsan, better known as "606," is prepared from *p*-hydroxyphenylarsinic acid, which is converted into the nitro-derivative and then reduced, when it gives the dihydroxydiaminoarsenobenzene.

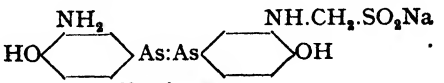


p-Hydroxyphenylarsinic acid. *m*-Nitro-*p* hydroxyphenylarsinic acid.



Dihydroxydiaminoarsenobenzene.
(Salvarsan.)

Neosalvarsan is prepared from salvarsan by adding an aqueous solution of formaldehyde sodium hydrosulphite or formaldehyde sodium hydroxysulphonate, $CH_2(OH)SO_3Na$, to an aqueous solution of salvarsan. It has the advantage of being readily soluble in a solution of sodium carbonate, and therefore more readily applied; but its therapeutic effect is similar to that of salvarsan. The formula is the following:

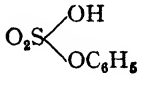


Neosalvarsan.

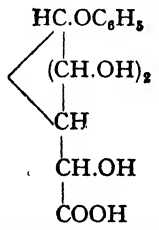
Organo-antimony compounds.—Many organic antimony compounds, such as the salts of antimonyl tartrate and substances analogous to salvarsan and its derivatives, have been examined

in connection with protozoal disease, but they do not compare favourably with those derived from arsenic.

The Elimination of Foreign Organic Compounds from the Body.—The general function of the living organism is to convert toxic substances into less poisonous products. The process takes place either by oxidation or reduction of the substance, or by the union of the substance or its oxidation or reduction product with some chemical compound supplied by the organism, such as glycuronic acid (p. 36) or glycine (p. 70). Thus benzene is oxidised to ordinary phenol and to catechol together with a little quinol, and the phenols, thus formed, are eliminated in the urine as phenyl sulphuric acid, or its sodium salt, or as phenyl glycuronic acid:

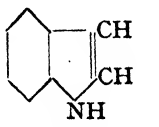


Phenyl sulphuric acid.

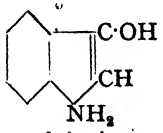


Phenyl glycuronic acid.

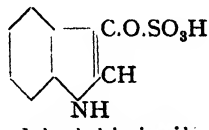
Indole, in the same way, is converted into indoxyl and is excreted as indoxylsulphuric acid (indylsulphuric acid):



Indole.



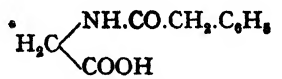
Indoxyl.



Indoxylsulphuric acid.

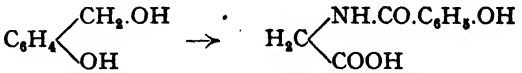
Toluene, benzyl alcohol, and benzaldehyde, are oxidised to benzoic acid, which, in union with glycine, is removed as hippuric acid (p. 74):

Hippuric acid.



Phenaceturic acid.

Other aromatic alcohols and acids behave similarly. Phenyl ethyl alcohol, $C_6H_5.CH_2.CH_2.OH$, is oxidised to phenylacetic acid, which then forms phenaceturic acid; saligenin becomes salicylic acid and then salicyluric acid:



Saligenin. Salicyluric acid.

Other cases of oxidation are the conversion of methyl alcohol to formic acid and aniline to *p*-aminophenol (p. 136).

An example of reduction is that of nitrobenzene, which is converted into aniline in passing through the system.

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